Testing agricultural impacts on breeding ground food resources as a driver of cuckoo population decline

Submitted by Lowell John Mills to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences, March 2019

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other university.

“The first picture of you,

The first picture of summer,

Seeing the flowers scream their joy.”

- The Lotus Eaters (1983)

Abstract

The common cuckoo *Cuculus canorus* has undergone a striking divergence in population trend between UK habitats since the 1980s. The breeding population in Scotland – in largely semi-natural open habitat – shows significant increase whereas there has been a significant decline in England. Here breeding numbers have remained stable or increased in semi-natural habitats, while woodland and farmland populations have plummeted. As a brood parasitic bird with a long-distance annual migration, the cuckoo has a unique network of relationships to songbird 'hosts', prey and habitat; and a disconnection between adult and nestling ecology due to lack of parental care. This thesis investigated the role of breeding ground land-use factors in driving cuckoo population decline. In the first chapter information was synthesised from the literature on potential threats and environmental impacts facing cuckoo populations, which also highlighted knowledge gaps and a basis for hypotheses in later chapters. In chapters 3 and 4 I investigated land-use and habitat influences on the nestling ecology of the cuckoo and a key host the meadow pipit *Anthus pratensis* at field sites in Dartmoor, Devon, UK. I assessed provisioning behaviour at unparasitised nests of meadow pipit, and used this baseline to test how host provisioning differed between host broods and cuckoo nestlings and fledglings, as indicators of how resource requirements differ between cuckoo and host in a relative stronghold habitat. There was evidence that host foraging habitat selection and investment in provisioning per unit time were similar between raising a cuckoo nestling and a host brood; but the nestling and fledgling periods were longer in cuckoos and the rate of provisioning was higher for cuckoo fledglings. Pipits also provided cuckoos with different diversity and frequency of prey taxa, further indicating that cuckoo nesting success requires different resources to that of unparasitised nests. In chapters 5 and 6 I focused on the diet of adult and juvenile cuckoos. In the first application of DNA sequencing to the study of cuckoo diet, adults in a relative stronghold habitat consumed large moth caterpillars (Lepidoptera) but frequently consumed Orthoptera and some Diptera families not previously reported as important prey. Analysis of moth capture data in Devon suggested some key prey species have declined even in semi-natural upland areas. I conclude with analysis of key findings including how they direct future research and conservation.

Contents

List of tables and figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

1. The ecology of common cuckoos *Cuculus canorus* increases populations' vulnerability to environmental change

The common cuckoo *Cuculus canorus* is an obligate avian brood-parasite. Females lay single eggs into 'host' songbird (Passeriformes) nests, where the hatchling is raised through to fledging of young by the deceived host 'foster' parents. The cuckoo also undertakes long-distance migration between the Palaearctic and the Afrotropics. Its migratory and breeding strategies mean the cuckoo has complex ecological interactions, influenced by both breeding and non-breeding period factors over a huge geographic area. Breeding season factors include the intrinsic role of the behaviour and ecology of the host (some species of which are also migrant) in influencing cuckoo breeding success and juvenile survival. Prey availability is also key to cuckoo breeding, with evidence that cuckoos feed for much of their daily activity after arrival from migration, to enter suitable condition to breed. On current evidence, prey is exclusively animal and chiefly invertebrates. Populations of invertebrates and nesting by songbirds are well documented to be vulnerable to changes in environment or anthropogenic land use, therefore patterns in cuckoo breeding population trends may relate to breeding ground land-use change such as agriculture. While recent advances in tracking migration and non-breeding habitat use have improved our understanding of the annual cycle, including stages with high mortality, up to date research of breeding ground ecology and sympatric drivers of change has received less attention. Understanding of all stages, including migratory connectivity, is needed to identify or rule out carry-over effects, wherein a factor at one stage has visible effects only at a subsequent stage.

In general, research of a declining bird species requires information on its natural history, the study of many individuals and populations, knowledge of demographic rates, and data on these factors plus external factors over sufficient time periods to span population stability and decline (Green 1995). As a brood parasite, demographic rates such as clutch size, hatching, fledging and productivity per adult cannot be conventionally measured in the cuckoo. Basic natural history elements such as adult diet are under-studied, and historically

centered on anecdotal field observation. Stomach contents studies have also featured but these inherently represent short term snapshots of diet, and carry greater ethical consideration and small sample sizes (often of incidentally deceased specimens) in modern studies. Historically, research on the cuckoo in the breeding range is rooted predominantly in questions surrounding brood parasitism and the evolutionary 'arms race' between host and parasite; but key questions such as specificity of cuckoo individuals and populations to hosts and habitat are still not fully understood. In this review, I provide a critical analysis of the knowledge of cuckoo ecology and how breeding range factors impact cuckoo populations. I also highlight current shortfalls in our understanding of this. The following specific questions are addressed. i) What are the patterns of cuckoo population trend in the breeding range? ii) What are the hypotheses regarding drivers of cuckoo decline, and what further vulnerabilities to environmental change or causes of decline can be identified from existing research on cuckoo and other migrants' and parasites' breeding ecology? iii) What are the priority research areas regarding the common cuckoo to ultimately inform conservation action?

i) What are the patterns of cuckoo population trend in the breeding range?

Globally the cuckoo is considered Least Concern by the IUCN (Birdlife International 2016). However, the combined trend in cuckoo abundance across 27 European countries shows a decline of 30% between 1980 and 2016 (PECBMS 2019). National trends in cuckoo abundance vary across Europe (mapped by Denerley 2014). Long term declines have been reported from distinct regions of Europe, for example the UK and the Netherlands (Harris *et al.* 2018, Boele *et al.* 2018), Denmark, Norway and Sweden (Moshøj *et al.* 2018, Kålås *et al.* 2014, Green *et al.* 2018), Hungary (Szép *et al.* 2012) and the Catalonia region of Spain (ICO 2018). Most national population monitoring projects reporting stability in cuckoo abundance are in Eastern Europe, including Estonia (Kuresoo *et al.* 2011), Latvia (Auniņš 2018) and Poland (Chodkiewicz *et al.* 2016). Cuckoo abundance has undergone a significant long term increase in Scotland in the UK (1995-2017, Harris *et al.* 2018) and

increase in Finland (1986-2012, Laaksonen and Lehikoinen 2013). Across the species' global range, the longest national trend in adult cuckoo population is the British Trust for Ornithology (BTO) Common Birds Census (CBC, 1962-2000) which was targeted towards farmland and woodland in England, and the Breeding Bird Survey (BBS, 1994 to present) that comprises two visits per breeding season to stratified random 1 km^2 survey squares across the UK. Analysis of combined data from the two surveys show that following relative stability in the 1960s and 1970s, England's cuckoo population declined steeply after 1985, with an overall decrease of 75% between 1967 and 2014 (Massimino *et al.* 2017).

The BBS shows contrasting cuckoo population trends for years 1995-2017 between Scotland, England and Wales (Harris *et al.* 2018) (Fig. 1). The trends captured by the BBS reflect the spatial trends in UK cuckoo population change recorded independently by successive bird atlases (Sharrock 1976, Gibbons *et*

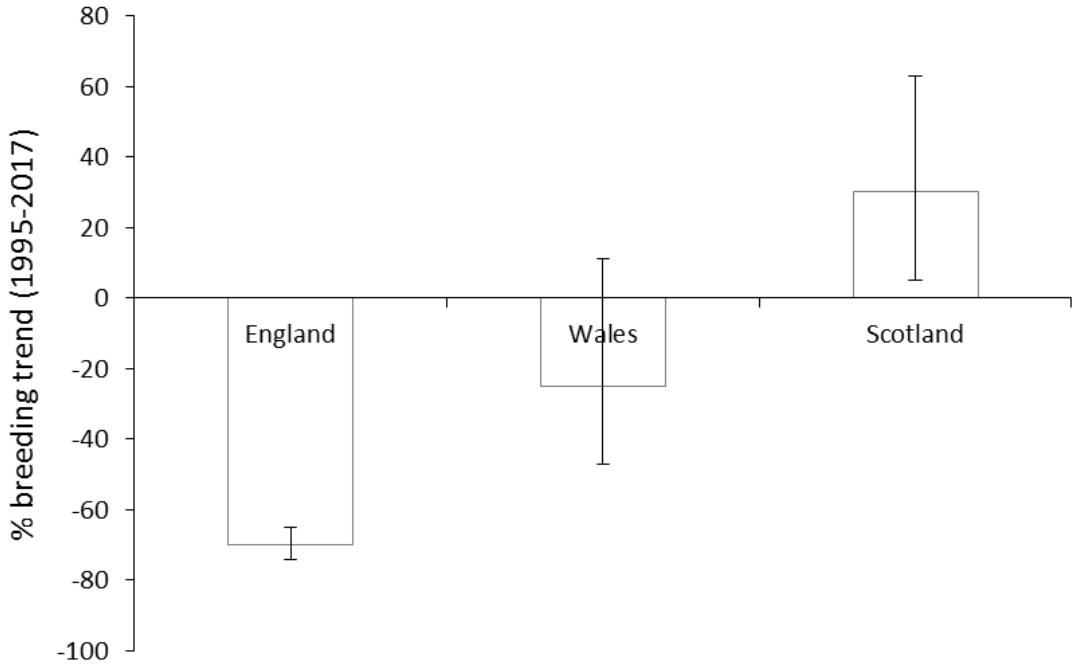


Figure 1. Mean percentage trend for period 1995 to 2017 in numbers of adult cuckoo *Cuculus canorus* counted by volunteer surveyors in stratified random 1 km squares in England (minimum annual n = 501), Wales (minimum n = 61) and Scotland (minimum n = 81). Counts conducted for the British Trust for Ornithology (BTO) Breeding Bird Survey (BBS). Error bars show 95% confidence intervals. Data originally published in Harris *et al.* (2018).

al. 1993, Balmer *et al.* 2013). BTO Bird Atlas fieldwork covers almost two orders of magnitude the land area of the BBS, over 4-5 years per atlas. Most 20 km squares in Britain reporting stability or increases in cuckoo abundance between BTO atlases are in Scotland. Cuckoo abundance has declined across large proportions of England and Wales (Balmer *et al.* 2013). Squares in England and Wales that showed increases in cuckoo abundance were located in areas with extensive semi-natural habitat (Balmer *et al.* 2013).

Considerable contrasts are also evident from studies which analysed BBS population trends in the UK by habitat type. The cuckoo showed the greatest difference in annual trend between habitats out of all conservation-concern species in the initial study (Newson *et al.* 2009). The most recent habitat-specific UK trends show breeding numbers were stable or increased between 1995 and 2011 in semi-natural lowland grass or heath, in contrast with non-significant decline in coniferous woodlands, and statistically significant declines in all other sufficiently covered habitats (population trends in upland habitats above 300 m altitude and in wetlands were not calculated from the BBS due to insufficient annual samples of squares containing these habitats) (Massimino *et al.* 2017). From comparison of proportional use of habitat types by cuckoos with their relative availability (Jacob's Preference Index), long-term declines are equally evident in habitats towards which cuckoos show preference and avoidance (Newson *et al.* 2009). This suggests decline is driven by larger-scale environmental or non-breeding factors (Newson *et al.* 2009).

ii) Review of hypotheses and mechanisms of population drivers

With its brood parasitic reproductive strategy, lack of ties to a nest site and suggested specialisation on large invertebrate prey (reviewed Wyllie 1981), the ecology of the common cuckoo includes associations with sympatric bird species and their resources of habitat and food that are seldom seen within the Palaearctic avifauna. While cuckoo declines are well documented in many regions of the breeding range, the causes are unclear. This is partly due to a poor understanding of how the cuckoo's combined peculiar attributes of brood parasitism, long-distance migration, and reliance on invertebrate prey, affect

sensitivity to environmental changes such as in climate or land-use. To assess how brood parasitism may affect vulnerability to such changes, Ducatez (2014) analysed IUCN conservation status and IUCN-reported population trend direction of species in five bird families containing one or more brood parasites, and reported that the brood parasitic species displayed a higher proportion with 'least concern' status and a lower proportion with 'decreasing' populations, than species with parental care. However the study made limited acknowledgement of varying research effort between species, IUCN methods of determining population change, or thresholds for 'data deficient' categorisation of species, within its main analysis. Additionally, although migratory or non-migratory status was considered in the above analysis, no distinction was made between short or long-distance migrants. Long-distance migrants show a higher proportion of species in decline (Hayhow *et al.* 2014), and lower flexibility to adapt phenology to that of their invertebrate food source (Both & Visser 2001), than both short-distance migrant and resident birds. The largest British landbirds that feed exclusively on invertebrates (common cuckoo, green woodpecker *Picus viridis*, European nightjar *Caprimulgus europaeus*, common swift *Apus apus*) show variation in British conservation status (Eaton *et al.* 2015): Knowledge of prey taxa of importance would assist in understanding whether this variation links to preferred prey (including specialist versus generalist), feeding habitat, or factors beyond prey availability.

Here, hypotheses and evidence regarding drivers of cuckoo population change are examined, divided into three broad groups of potential drivers, namely i) migration, ii) breeding ground hosts and habitat (framed by brood parasitism), and iii) breeding ground prey availability. This includes discussion of both existing hypotheses and new hypotheses arising from synthesis of research knowledge of cuckoo ecology.

MIGRATION

The cuckoo undertakes annual migration between the Palaearctic and southern Africa, for the Northern Hemisphere summer and winter periods respectively (Willemoes *et al.* 2014, Hewson *et al.* 2016, Vega *et al.* 2016, Bán *et al.* 2018).

This includes the first winter after hatching (Vega *et al.* 2016). Afro-Palaearctic migrants are considered to face 'multiple-jeopardy', reliant on resources at many locations spanning two continents, and potentially facing population-scale threats at all stages (Newton 2004a, Vickery *et al.* 2014).

Correlation of population trend with wintering area in migrants

Analyses categorising Afro-Palaearctic migrants according to bioclimatic zone in Africa where the non-breeding period is spent, have aimed to determine the importance of non-breeding area factors in driving population change in migrants. The cuckoo has generally been classified as overwintering in the 'southern' sub-Saharan zone, a region dominated by patchily wooded savannah, south of the 'humid' zone belt of rainforest of central Africa (Hayhow *et al.* 2014, Beresford *et al.* 2018 supplementary materials). Mean location of tracked birds in Northern Hemisphere mid-winter was at the edge between these two zones (Hewson *et al.* 2016, Williams *et al.* 2016) so in some cases the cuckoo is classified into the humid zone wintering group (Ockendon *et al.* 2012). Bird species wintering in both of these zones stage in the sub-Saharan 'arid zone' (the Sahel) to the north, while another assemblage overwinters in this region. Earlier analyses suggested species overwintering in the humid and southern zones had collectively more negative breeding population trends than those overwintering in the arid zone (Ockendon *et al.* 2012, Atkinson *et al.* 2014, Ockendon *et al.* 2014). Most recent analyses suggest that species with the most negative population trends are those overwintering in the drier regions (southern zone and northern Sahel), especially in grasslands (Beresford *et al.* 2018). Overall these studies concur that **the cuckoo is part of a wintering migrant group with relatively more negative breeding trends in western Europe.**

The mechanisms of how the bioclimatic wintering zone may influence breeding populations remain unclear. The geographical stages of importance to population decline in migratory birds are most identifiable in species for which i) staging areas are known and consistent, and ii) population and demography are recorded at as many of these locations as possible. This has longest been the case for waterfowl, studies of which gave early insight into migratory

connectivity and carry-over effects, but for the cuckoo only breeding ground adult abundance, or presence-absence, are consistently monitored. While tracking technology advances have improved our knowledge of migration routes and staging locations for many species (e.g. Åkesson *et al.* 2012, Bairlein *et al.* 2012, Cresswell & Edwards 2013, van Wijk *et al.* 2013, Lormee *et al.* 2016) including the cuckoo (Willemoes *et al.* 2014, Hewson *et al.* 2016, Vega *et al.* 2016, Bán *et al.* 2018), analysis of non-breeding effects in almost all Afro-Palaearctic migrants remain limited by lack of detailed knowledge of distribution and habitat use in non-breeding areas. This is as true for European staging areas as those in Africa, with some staging areas in Europe only recently linked to specific breeding populations through PTT (platform transmitter terminal) tracking of adult migration (e.g. Hewson *et al.* 2016). Such findings, in addition to furthering core knowledge of migratory connectivity, provide bases on which to initiate research collaboration and conservation partnerships that are vital for effective conservation of long-distance migrants.

Continued use of tracking technology can form only part of future research effort into non-breeding region drivers of migrant bird populations. **Following confirmation of migratory routes and staging regions, study at multiple locations along the annual route is required** concerning abundance, demography (survival rates, age and sex ratios), body condition and ecology of birds when using the specific region. These studies are needed to highlight the identity and location of driving factors, and identify key habitat and food resources at each stage (Marra *et al.* 2015). This often requires *in situ* field study of migrant birds at non-breeding stages (e.g. Blackburn and Cresswell 2016). However, 'signature' ratios of stable isotopes (of deuterium, carbon, nitrogen) deposited into keratinous tissue (such as feathers and claws) at known stages of the annual cycle have been used effectively to infer and link geography and resource use at previous stages along migratory routes (Marra *et al.* 1998), and have been used to study ecology at migratory stages remotely (e.g. Bearhop *et al.* 2004). The increasing accuracy and variety of data that can be collected and transmitted by tracking technology is also improving capability to study animal ecology remotely, and non-breeding home-range size in the cuckoo has already received study by this means (Williams *et al.* 2016). From

locations transmitted from tracked birds on migration and at the overwintering sites, Willemoes *et al.* (2014) inferred that southbound stopover sites in Europe and Africa were mainly open farmland and wetlands, compared to more forested areas during overwintering and northbound staging. However, much of the research coverage recommended above has not yet been attempted for the cuckoo away from the breeding grounds.

Correlation of population trend with migration route in cuckoos

Tracking of adult cuckoos breeding in Britain has shown correlation between migration route and regional breeding population trend (Hewson *et al.* 2016). Both mortality and regional population trend differed between groups of tagged adult male cuckoos taking two distinct southbound migration routes (Hewson *et al.* 2016). PTT tagged birds tracked from a range of localities over several years took either an eastern route via Italy and the Balkans, or a western route via Iberia, to sub-Saharan Africa, with individuals selecting a consistent route in successive years. Birds taking the western route left Britain later than eastern route birds, and had a significantly lower survival rate by the time they had completed the Sahara desert crossing despite their shorter migration distance. Mortality during this phase was concentrated in Europe rather than during the desert crossing. Survival rates were similar between the two route groups through the remainder of the non-breeding period where the groups undertook similar staging and routes. The proportion of birds using the western route varied between the study sub-populations, and this significantly correlated with magnitude of regional population decline from two independent survey datasets. However these proportions were calculated from small numbers of tagged birds (mean regional sample n = 4.67).

While there is discussion of the role of drought and wildfire in south-west Europe in causing the reported mortality of birds using this route (Hewson *et al.* 2016), the study ultimately acknowledges that the difference in migration route also entails a difference in departure date; and with this a difference in the proportion of fueling that takes place in the breeding grounds as opposed to southbound stopover sites. Furthermore, there is reference in this study to particularly severe declines in prey moth (Lepidoptera) species in regions where

western-route birds were breeding (and tagged). With the potential for mortality in southern Europe to equally relate to a carryover effect from differing departure date and fueling conditions, the role of factors present along the western migration route itself cannot be ascertained, and this reinforces the need for ecological study across the entire annual range of the cuckoo and other long-distance migrants.

While a number of tracking studies of common cuckoos have been published before and since, the study by Hewson *et al.* (2016) is the only study in the literature to refer to the conservation status of its study populations. This may be due to the global Least Concern status of the cuckoo, or its variable conservation status between European countries. Routes taken over the Mediterranean Sea and Sahara by all tracked adults from southern Sweden and northern Denmark (Willemoes *et al.* 2014), Hungary (Bán *et al.* 2018) and northern Norway and Finland (Vega *et al.* 2016) resemble the eastern route as defined by Hewson *et al.* (2016). While trends in cuckoo abundance from national monitoring programmes are available for these countries, they may not reflect the regional or local trends of these study populations. Reference to population trend at a local or regional scale is recommended in future research on the common cuckoo, given previous observations that population trend and various aspects of the ecology appear to differ between habitats and host species.

Non-breeding dependence on seasonal weather and vegetation greening

In contrast to during southbound migration, tracked birds from different breeding populations show similar northbound migration timing, staging and routes (Willemoes *et al.* 2014, Hewson *et al.* 2016). Staging by cuckoos tracked along their annual migration route correlated spatio-temporally with high vegetation greenness index NDVI (Normalized Difference Vegetation Index) (Thorup *et al.* 2017). This suggests **cuckoo movements in the non-breeding period represent birds tracking (invertebrate and water) resources associated with greening of vegetation**. Movements also closely reflected those of other large insectivorous migrant birds, further supporting the role of food availability in determining these movements (Jacobsen *et al.* 2017).

In central Africa, seasonal greening is heavily associated with oscillation of the inter-tropical convergence zone (Schneider *et al.* 2014, Beresford *et al.* 2018). Projections for future shifts in NDVI patterns suggested a delay in greening at relevant cuckoo staging regions in the (Northern Hemisphere) autumn (Thorup *et al.* 2017). **This mismatch between cuckoo and staging ground phenology would mean arrival to lower primary production and potentially fewer prey if cuckoos were unable to shift arrival later in autumn to synchronise** (Thorup *et al.* 2017). Worryingly, tracking studies indicate both that autumn is already a period of heightened pressure on cuckoos, with southbound migration through Mediterranean regions accounting for most mortality in tracked birds, and that individual flexibility in migration timing may be too limited to allow adjustment for a shift in NDVI timings (Hewson *et al.* 2016). It has also been suggested that if vegetation greening events occur later or further south, this could delay resource-tracking migrants such as cuckoos' return to breeding grounds, or see them leaving sub-Saharan Africa in poorer condition (Beresford *et al.* 2018). In particular, western Africa appears essential to the cuckoo and many other southerly-wintering migrants, for staging in the early northern spring, ahead of relatively rapid migration to Europe (Jacobsen *et al.* 2017), with food supply likely to strongly relate to seasonal conditions (Beresford *et al.* 2018). Consistency of tracked migration between birds suggests the entire European or perhaps Palaearctic population of common cuckoo rely on this region (Willemoes *et al.* 2014, Hewson *et al.* 2016).

Inflexibility of cuckoos as long-distance migrants to synchronise arrival with advancing spring phenology

Spring events are occurring progressively earlier in temperate regions of the Northern Hemisphere due to increasing mean temperatures (Parmesan & Yohe 2003, Root *et al.* 2003). In Europe, spring phenology has advanced by mean 2.5 days per decade since 1970 (Menzel *et al.* 2006). There has been particular interest in how this could impact on passerine bird species whose breeding is often synchronised with mass emergence of invertebrate prey provisioned to their nestlings (reviewed by Both 2010). Population trends for the period 1990-2000 were shown to be significantly more negative for migrant bird species which had not advanced their arrival date in Europe (based on multiple time

series of ≥15 years, over total period 1960-2006, Møller *et al.* 2008). This suggests that failure to maintain synchrony with phenology of breeding ground resources could drive population declines.

If similar shifts in conditions or phenology have not taken place in cuckoos' western African northbound staging area, or are not used as cues for departure from this area, then cuckoos may be unable to behaviourally advance their departure to arrive synchronously with key breeding ground phenology (Lehikoinen *et al.* 2004). In cuckoos this could include phenology of prey (e.g. emergence of overwintering moth larvae) and also host nests (peak egg-laying period). Examples of cues that influence migration departures of other species include photoperiod (suggested as a key initial cue by Berthold 1996) and food availability tied to tropical seasonality (as demonstrated in American redstarts *Setophaga ruticilla*, Studds & Marra 2011). Arrival and departure timing of migrant birds can alternatively change through evolutionary selection, if individuals arriving at earlier or later dates confer a survival or reproductive advantage (Lehikoinen *et al.* 2004).

Analyses of cuckoo arrival dates in Europe suggest that **in most regions cuckoo arrival dates have advanced significantly less than general spring phenology, and phenology of resident and short-distance-migrant host bird species.** A mean of 10.0 days advancement has been measured across a range of spring phenological events over 40 years (Menzel *et al.* 2006). Multiple time series of first arrival dates of birds in breeding habitat across Europe suggest short-distance migrant hosts have advanced by mean 14.6 days and long-distance hosts 6.0 days over 40 years. In comparison, cuckoo arrival has advanced by mean 5.3 days (Saino *et al.* 2009). Advancement in cuckoo arrival date in Britain is visible and significant over the timesecale of centuries (Sparks 1999, Davies 2015), but not over recent decades (Davies 2015), though arrival dates do show correlation with spring temperature (Sparks 1999). Species such as the cuckoo and turtle dove *Streptopelia turtur*, which have not shifted their timing of migration, have more negative population trends (1960s-2010) than migrants that have shifted their migration timing and now spend a longer period of the year in the UK breeding grounds (Newson *et al.* 2016, Hayhow *et al.* 2017). Cuckoo arrival to Arctic Norway has reportedly advanced roughly 10

days in 32 years, mostly in the final 15 years (Barrett 2014), though arrival dates in this high latitude remain considerably later than in temperate Europe. **Research is urgently needed to identify cues or mechanisms of cuckoos departing the western African staging grounds on northbound migration, and what constraints exist on their ability to maintain synchrony with breeding ground phenology**.

BREEDING GROUND 1 – HOSTS AND LAYING HABITAT

Across its global range many bird species are parasitised by the cuckoo. However, individual females lay eggs in one habitat and specialise in parasitising one or a few host species relative to all habitats and hosts in the vicinity. Here, I discuss the hypotheses of impacts on cuckoo populations in the context of brood parasitism and this specificity to habitat and host.

The threat of reduced host nests due to advancing host egg-laying dates

The vulnerability of the cuckoo, as a long-distance migrant, to advancing spring phenology in the breeding grounds is compounded by its niche as a brood parasite. Cuckoo eggs are laid during the host nest's laying period (Baldamus 1892, Rey 1892). Cuckoos arriving from migration and reaching body condition for egg-laying ahead of peak availability of host nests at the laying stage, is critical to breeding success and productivity. On this basis, widespread loss of synchrony with host nesting phenology would have population-level consequences for cuckoos.

Analyses at both a British (Douglas *et al.* 2010) and European scale (Saino *et al.* 2009) suggest that arrival and laying dates of resident and short-distance migrant host species (e.g. dunnock *Prunella modularis*, meadow pipit *Anthus pratensis*, common linnet *Linaria cannabina*, white wagtail *Motacilla alba*, European robin *Erithacus rubecula*) have advanced, causing reduced availability of these species' nests during the cuckoo laying period. Magnitude of change in use of resident and short-distance migrant hosts by cuckoos correlated negatively and significantly with change in regional spring temperature (Møller *et al.* 2011), further suggesting that this change is climate-

driven. Cuckoos also exploit some long-distance migrant hosts (e.g. reed warbler *Acrocephalus scirpaceus*) and availability of these species' nests is estimated to have remained stable or even increased (Saino *et al.* 2009, Douglas *et al.* 2010, Barrett 2014).

With both gains and losses therefore recorded in the 'host nest resource', it has proved difficult to establish whether mismatching phenology with some host species has significant consequences for cuckoo populations. Having identified phenological (arrival date) shifts for cuckoos, and host species of different migration distance groups, Saino *et al.* (2009) highlighted the myriad complexities in cuckoo and host biology that make predicting effects of mismatching phenology on cuckoo (or host) populations. In brief, these are that host population sizes, trends, and accuracy of data on these, are geographically variable; that cuckoos sub-populations are host specialist and host-specific parasitism rate varies geographically; that effects on availability of second clutches is complex and difficult to predict; and that arrival date shift of migrant hosts may vary in effect with how much their arrival date differs from that of cuckoos. In contrast, in addition to analysing phenological (first egg date) shifts of British hosts, Douglas *et al.* (2010) attempted to quantify impacts of phenological shifts on proportional availability of nests, and ultimately cuckoo abundance (using BBS data). Conclusions drawn by Douglas *et al.* (2010) do not take into account a number of the aforementioned complexities detailed in Saino *et al.* (2009). Cuckoo phenology was assumed not to have shifted in Britain, and incorrectly cited that evidence was equivocal for advancement in Europe. While evidence is lacking of linear advancement in cuckoo phenology in Britain, failure to include variation in phenology (e.g. using first arrival date at a consistent location) may have influenced the model findings. The cuckoo egg-laying period was instead maintained as a constant between 15 May and 30 June, when this is instead likely to correlate with arrival date (Saino *et al.* 2009), and additionally analysis of nest record cards show that cuckoo egg-laying periods vary with host species targeted (Lack 1963, Rose 1982). Furthermore, cuckoos show specificity towards one or few host species. The use of national abundance of cuckoos (from the BBS dataset) as a response variable by Douglas *et al.* (2010) when modelling the effects of abundance or phenology of

single host species, was therefore incapable of capturing effects on the specific sub-population of cuckoos that utilises this host. Although this was acknowledged by the authors themselves (Douglas *et al.* 2010) it is nonetheless a significant source of type II error (i.e. false negative) in the study. There was also limited reference to known variation in population trend of hosts and cuckoos that exist between habitats (Massimino *et al.* 2017); and that species targeted by cuckoos as hosts in some sites or habitats are not targeted in others, due to spatial variation in host specificity in cuckoos (Saino *et al.* 2009). Repeating the analyses for each host species to include only the geographic area in which the host is known to be targeted by cuckoos could account for specificity but would likely reduce sample sizes to the point of unviability. Using the BBS dataset limited the timescale of the Douglas *et al.* (2010) study to 1994 to 2007, considerably shorter than the mean 37 years covered by analyses in Saino *et al.* (2009). This may explain why the phenological shifts reported in resident and long-distance migrant species were similar in Douglas *et al.* (2010), compared to significantly greater shifts in phenology for short-distance than long-distance migrant hosts in Saino *et al.* (2009). Using data from the BTO Nest Record Scheme (1939 to present) instead of the BBS, plus cuckoo arrival dates, to estimate changes in host and cuckoo phenology, and changes in population size of cuckoos parasitising nests in different hosts and habitats – as attempted in part by Brooke and Davies (1987) and Lindholm (1999) – would permit a longer timescale and could better account for host specificity of cuckoos.

Despite limitations to the correlative analyses between hosts and cuckoos by Douglas *et al.* (2010), a notable finding is that first egg date has not significantly shifted in meadow pipits, the main cuckoo host in British uplands and therefore a key host to remaining cuckoo populations in Britain. This is concurrent with longer-term analysis of meadow pipit laying date trends in Britain (Massimino *et al.* 2017) and of arrival date of meadow pipits in north Norway where they are also the main cuckoo host (Barrett 2014). The meadow pipit is a partial short distance migrant in Britain and effectively a long-distance migrant to north Norway. At higher altitudes where temperature changes are more sensitive to heat energy input, and air, clouds and storms are more rapidly transported

(Whiteman 2000) weather is more rapidly changeable and phenology of vertebrates may be less specifically responsive to spring temperature. From data on nesting phenology in Britain and northern Norway, availability of meadow pipit nests to cuckoo parasitism (as a percentage of all breeding attempts a year) is indicated not to have significantly changed (Douglas *et al.* 2010, Barrett 2014), and from phenology data from Britain the availability of reed warbler nests is indicated to have increased (Douglas *et al.* 2010). This may explain why reed warbler and meadow pipit remain common hosts of the cuckoo in Britain (Brooke & Davies 1987). Both host species show less regional and habitat variation in breeding population trend than the cuckoo (Massimino *et al.* 2017), although this is partly because the national populations of both species are associated with a narrower range of habitats than the cuckoo.

In summary it has been possible to infer with the available data that phenology of cuckoo host nests has changed in Europe, that climate is likely to have driven this, and that proportion of annual host nest attempts accessible to cuckoos has changed (Saino *et al.* 2009, Douglas *et al.* 2010, Møller *et al.* 2011). This change in access has been estimated to be positive and negative dependent on host species, and may relate largely to whether the host is also a migrant like the cuckoo (Saino *et al.* 2009, Douglas *et al.* 2010, Barrett 2014). Determining whether, and to what extent, the change in access to host nest attempts impacts on cuckoo populations, is made challenging by geographic variation of cuckoo and host ecology, and variation in cuckoo demography with host species (Saino *et al.* 2009). However, studies that account for these factors may be possible through creative and cautious use of nest record card scheme and migrant arrival date datasets. In the context of diagnosing and halting cuckoo population declines in Europe, studies of this kind are urgently needed.

The threat of loss of specific breeding habitat

Across the global range many bird species are parasitised by the cuckoo (Glue & Morgan 1972, Wyllie 1981, Moksnes & Røskaft 1995, Campobello & Sealy 2009). All hosts documented to host a cuckoo egg and successfully raise a cuckoo nestling have been songbirds (Passeriformes) which feed their own young an (invertebrate) animal diet. Suitable host species are present in most

habitats in the Palaearctic (Wyllie 1981, Cramp 1988). As a result, cuckoo breeding is documented widely in habitat and altitude (from sea level up to 3800m, Birdlife International 2016). In Britain alone, the cuckoo has historically been recorded from coastal habitats, through woodland and agricultural land, to upland grass and moor habitats (Glue & Morgan 1972). With this broad range of breeding habitats, the cuckoo has long been classified as a habitat generalist. However, individual females lay eggs in one habitat and specialise in parasitising one or a few host species relative to all habitats and hosts in the vicinity (Baldamus 1892, Rey 1892, Chance 1940, Wyllie 1981, Nakamura & Miyazawa 1997, Marchetti *et al.* 1998, Vogl *et al.* 2002, Skjelseth *et al.* 2004, Fossøy *et al.* 2011). Adoption of an entirely new host has only observably taken place within cuckoos' existing laying habitat, as opposed to cuckoo expansion into new laying habitat (Nakamura 1990). For example, female cuckoos parasitising reed warblers are predicted to readily switch to targeting great reed warblers *Acrocephalus arundinaceus* (Kleven *et al.* 2004) but these hosts use the same habitat. These observations suggest that **female cuckoos are constrained to one laying habitat type and a narrow range of host species**. **On this basis, it is possible for the removal of one habitat type to result in the loss of all suitable breeding (host nest finding and egg-laying) habitat for the local cuckoo population, even if other habitat known to be used by cuckoos elsewhere in the global range is present.** Host or habitat specialism at the local population level may partition cuckoos living in sympatry from interacting. Understanding the level of specialism and isolation present in cuckoo populations is key for conservation of the species.

The evolutionary aspect of specialising on primary host species, how this is maintained, and what the resultant groupings of cuckoos using the same host constitute (maternal lineages, 'host-races' a.k.a *gentes*, or in fact cryptic species?) is beyond the scope of this review but has been summarised elsewhere (Fuisz & de Kort 2007, Moksnes *et al.* 2013). One key question in this area, of relevance to cuckoo conservation ecology, is whether host specialism is maintained through adult females (and possibly males) undertaking breeding behaviour in habitats or host species which match the habitat or host in which they were raised as a parasitic nestling. If this is the

case, this is predicted to constrain cuckoo populations even further with regard to habitat suitability and the capacity for populations to adapt to environmental change, as inflexibility in the habitat or host a cuckoo can target for brood parasitism would persist in successive generations, increasing pace of local extinctions (Lindholm 1999). Small numbers of marked juvenile cuckoos have been re-found at their natal site (Wyllie 1981), but there is no direct evidence that young cuckoos parasitise primarily their natal host species, or commonly lay at their natal site or habitat type in adulthood. However, females parasitising many host species display mimicry in their egg colour and pattern to eggs of the host species (Baldamus 1892, Rey 1892). This suggests that females in successive generations parasitise the same (or very similar) host species, as egg mimicry would be most likely to evolve if a narrow range of host egg colour and pattern was exerting selection pressure over many generations. Whether this also qualifies as evidence that male cuckoos selectively breed in the vicinity of similar habitat and host species in successive generations, is dependent on how exactly cuckoo egg colour and pattern are controlled by genes; on i) the female-specific sex chromosome W that is only inherited maternally, and ii) autosomal DNA (Fossøy *et al.* 2011). If autosomal genes control egg pattern, paternal genes could influence egg pattern in female offspring and this scenario would select for mating between adults of matching target host to maintain successful egg mimicry.

Specialism in male cuckoos and implications for population viability

Do males also specialise, by mating only with females of specific host or habitat specialism? In studies of cuckoo populations in heterogeneous habitat with multiple host species present, genotyping of cuckoo nestlings found males to sire young in multiple host species' nests; while females preferentially targeted a single host species (Marchetti *et al.* 1998, Skjelseth *et al.* 2004). This would indicate that male cuckoos lack host specificity and are less constrained to a narrow range of breeding habitats than females. This would provide better conditions for gene flow between populations of females targeting different hosts, and higher overall availability of mates as a resource. However, a subsequent study in a mosaic of habitats in Bulgaria showed that males that were genotypically matched to young in multiple host species' nests (any two of

corn bunting *Emberiza calandra*, great reed warbler, and marsh warbler *Acrocephalus palustris*), nonetheless sired young in one host species' nest more than others (ratios of dominant to other host ranged 2:1 to 10:1) (Fossøy *et al.* 2011). This would indicate that males mate preferentially with females of a particular host-specialism, potentially through females or both sexes having preference to mate in habitat matching their natal habitat. Additionally, both male and female nestling cuckoos from eggs laid into reed warbler nests are responsive specifically to alarm calls of this host, even if experimentally transferred to the nest of a different host (Davies *et al.* 2006). This demonstrates a genetically inherited host-specific behaviour which is found in both sexes and therefore cannot be controlled only by genes on the female chromosome (Fuisz & de Kort 2007). This suggests that cuckoo nestlings have adaptations that are dependent on males assortatively mating with females targeting a specific host. **If both sexes are under selection pressure to focus breeding behaviour around a narrow range of habitats and hosts, cuckoo populations targeting different hosts in the same area could be genetically isolated.** This has implications for conservation as each regional population would in reality comprise multiple genetically isolated populations, and apparent mate availability would conceal considerably lower actual mate availability to each host-specialist group. This poses a greater risk of local extinction than if regional populations included frequent viable interbreeding between individuals of different host specificity. Declines have been recorded in genetically divergent bird populations separated primarily by different resource use, though reduced gene flow is considered only a potential threat to these populations rather than a confirmed cause of decline (Welch *et al.* 2012, Wiley *et al.* 2019).

In summary, female, and possibly male, cuckoos are specialist in breeding habitat and host in comparison to the species' overall generalist status. Individual specialism in ecology within population-level generalists has previously been highlighted, for example in seabird feeding strategy (Ramírez *et al.* 2015). Presence of specialist sub-populations potentially increases vulnerability of one or more sub-populations to environmental change, and could in turn influence population dynamics of the wider population (Bolnick *et al.* 2002, Araújo *et al.* 2011, Welch *et al.* 2012, Wiley *et al.* 2019). In the

common cuckoo, habitat specialism of local populations presents an obstacle to effective conservation, as knowledge of the targeted habitat type is required if suitable habitat is to be preserved. Removal of specialist breeding habitat, and preservation of habitat used by cuckoos elsewhere in the breeding range that is not locally targeted, could represent a complete loss of suitable local breeding habitat. Habitat or host specialism in both female and male cuckoos would limit the resource of prospective mates available to each individual in a population of varying specialist habitat or host, with implications for gene flow and population viability. There is direct evidence for males and females predominantly parenting young in the nests of one host (e.g. Vogl *et al.* 2002, Fossøy *et al.* 2011). However, the mechanism for how this is maintained, such as cuckoos targeting habitats and hosts that match those in which they were raised, assortative mating by females with males of a certain phenotype that indicates natal habitat such as call (Fuisz & de Kort 2007), will only be directly observable with miniaturisation and improved lifespan of tracking technology which will allow individuals to be tracked from nestling to adult stages.

Effect of host species on nestling development and juvenile survival

As a brood parasite, the ecology of the nestling cuckoo is framed by host ecology up to the point of independence. Measuring mass is the simplest approach to studying quality of developing nestlings, and data on cuckoo nestling mass has been collected in several studies (Bussmann 1947, Kilner *et al.* 1999, Kleven *et al.* 1999, Grim & Honza 2001, Grim 2006, Grim *et al.* 2017). Mass is a crude indicator of nestling nutrition compared to measuring intake of specific nutrients such as protein and lipids, but this requires study of prey intake and data on the nutrient content of each prey type. Few studies have accurately measured intake (Grim & Honza 2001, Martín-Gálvez *et al.* 2005) and none have additionally measured nutrient content of prey. The best means of simultaneously measuring food intake and sampling prey invertebrate specimens is the use of a temporary neck ligature to prevent food from being swallowed by the nestling which can then be collected (as in Grim & Honza 2001), but this approach carries significant ethical considerations especially in small and potentially vulnerable study populations. Video observation (as in Martín-Gálvez *et al.* 2005), with simultaneous collection of reference

invertebrate specimens (as demonstrated for species molecular identification purposes in King *et al.* 2015) is another option, but requires prey in videos to be identified and matched to specimens in the collection, which may be absent. From nestling mass studies, cuckoo growth rate and mass at fledging vary with host species. Nestlings grew significantly larger in great reed warbler nests than in reed warbler nests, including nestlings from eggs originally laid in reed warbler nests which ruled out any effect of great reed warbler nests being more commonly parasitised by higher quality cuckoos (Kleven *et al.* 1999). This suggests that growth rate is limited by host parental performance, as great reed warblers are a larger species which may provision larger prey loads to their nests. When experimentally placed into song thrush *Turdus philomelos* nests, cuckoo nestlings at fledging weighed almost as much as a captive cuckoo fed *ad libitum* (Bussmann 1947, Grim 2006). Mass at fledging varies with host species and is a key factor in juvenile survival (Magrath 1991, Ringsby *et al.* 1998, Medeiros & Freed 2009, Morrison *et al.* 2009), suggesting that **the cuckoo population is subject to host-specific differences in juvenile survival across its global range**. Host selection varies with habitat, so juvenile survival is expected to be influenced by habitat, and factors influencing habitat loss such as land use change.

Body mass of juveniles is lower than that of adults, both upon reaching independence from the host (Wyllie 1981) and when captured at ringing sites in the UK, apparently including birds on migration (Seel 1977). Wyllie (1981) speculated that juveniles substantially gained weight before leaving southern Europe, but no data was available to support this. Migration of tracked juvenile cuckoos from Finland involved stopovers in Europe (Vega *et al.* 2016). This suggests feeding to reach adult mass and fuel for migration takes place after leaving the natal region. Taken together with variation in fledgling mass as discussed above, juvenile cuckoos are indicated to be under dual pressure of commencing migration with lower body mass than adults, and this mass varying between natal host species. Survival rates of migrating juvenile birds are typically lower than those of adults, even when they are similar in mass to adults (e.g. Oppel *et al.* 2015). On this basis there is substantial variation in juvenile survival between natal host species, and juvenile survival is lower

compared to adult survival than in many other migrant species. This has potentially dramatic implications for maintaining sufficient recruitment to breeding cuckoo populations, as adult survival may itself be relatively low in cuckoos due to the long migratory cycle (Hewson *et al.* 2016).

Identifying nestling diet is of high importance to cuckoo conservation biology. Wyllie (1981) concluded that hosts feed the cuckoo nestling the same diet as that fed to their own nestlings, but without any observational evidence to support this (Grim & Honza 2001). This might be assumed on the basis that foster parents are deceived that the cuckoo nestling is their own offspring. However, the nestling cuckoo at later stages of development is larger and heavier than an entire host brood, the cuckoo nestling period extends significantly beyond the typical 11-13 days spent by host nestlings before fledging, and furthermore the fledgling cuckoo can successfully solicit host provisioning for several weeks after fledging (Wyllie 1981). Nutritional requirements of raising a cuckoo are therefore greater than those of a whole host brood, and there has been interest in whether host provisioning behaviour is influenced by the nestling's adaptive signalling to foster parents, or its excessive size at later stages.

While mass of food fed to a nestling by host 'foster parents' per hour was found to be consistently higher for a cuckoo compared to (single) host nestlings under field-based experimental conditions (Grim & Honza 2001), observational studies suggest host provisioning visit rates to cuckoo nestlings are not significantly different to those to host broods (Kilner *et al.* 1999). The question of whether host provisioning rate differs when raising a cuckoo is still not properly addressed, as while previous studies have measured visit rate and occasionally prey load size, no study has accounted for whether foraging distance or habitat use differs for hosts when provisioning a cuckoo.

Significant differences in diet composition between cuckoo and host nestlings could indicate a difference in cuckoo ecology from host ecology, and that cuckoo breeding populations may not be sufficiently protected by conservation of host nesting populations. While Brooke and Davies (1989) generally found little difference in range of invertebrate prey, or frequency of different prey size

classes, between cuckoo and reed warbler nestlings, beetles (Coleoptera) have often occurred in greater frequency at parasitised nests than host nests (Brooke & Davies 1989, Grim & Honza 2001, Martín-Gálvez *et al.* 2005). Cuckoos were also fed a larger volume of the smallest prey taxa (e.g. aphids), particularly after the cuckoo nestling's mass increased beyond that of a whole host brood (Grim & Honza 2001). The indication that hosts may forage less selectively for large cuckoo nestlings than for their own nestlings, has implications for cuckoo nestling survival. Is it a sign that hosts struggle to forage sufficient prey in late stages of cuckoo development? Insectivorous hosts of even a very small body mass have successfully raised a cuckoo to fledging (Wyllie 1981), but do cuckoos leave the nest of some hosts in relatively poor condition? This could be examined across cuckoos raised by different hosts, but body condition is not readily compared between cuckoo and host nestlings due to structure and size differences between species.

Requirement of additional habitat features to succeed in brood parasitism

Further to hosts and host nesting habitat, tracking and nest monitoring studies indicate the importance of perching positions to cuckoo breeding habitat suitability. Numerous studies suggest parasitism risk to a host nest increases with proximity to trees suitable for perching (Alverez 1993, Øien *et al.* 1996, Honza *et al.* 1998, Clarke *et al.* 2001). While less experienced host pairs more vulnerable to brood parasitism may be relegated to edge locations in nesting populations through competition, these observations could indicate importance of perch availability as a vantage point for female cuckoos. Tracked female cuckoos occupied raised perches to watch and parasitise nests (Nakamura & Miyazawa 1997, Honza *et al.* 2002). Raised positions are also important for male cuckoo display and song (Wyllie 1981, Nakamura & Miyazawa 1997), and for foraging (pers. obs.). Although cuckoos may use natural changes in elevation (tall slopes and valleys) to gain a view over their breeding area, most commonly these behaviours require trees or similar perches, both natural and artificial (Wyllie 1981). Male song perches may equally be exposed or covered by foliage (Wyllie 1981). Females may preferentially use trees with extensive foliage that conceal them from hosts for nest watching (Mills *et al.* unpublished data). In standardised breeding bird surveys in uplands in England, cuckoos

were generally absent from the treeless plateaus which were rich in meadow pipit hosts (Geary 2000, Stanbury *et al.* 2006, Booker *et al.* 2017). Cuckoo presence at the 2 x 2 km scale correlated with woodland cover (Denerley 2014). There is anecdotal evidence that cuckoos may benefit from afforestation of open semi-natural heath and bog areas such as the Flow Country in northern Scotland (Zühlke 2009, Denerley 2014). The range of functions raised perches such as trees serve during cuckoo breeding lead us to suggest that in addition to host nests, breeding cuckoos require structures on which to perch in order to successfully reproduce.

Cuckoos upon leaving the nest are large and emit loud begging calls, but have poor flight capability for escaping predators (Wyllie 1981). Newly fledged birds therefore position themselves in tall herb or shrub vegetation for cover. More mature fledglings often relocate to trees or wooded habitat which can be several hundred metres from the nest and in habitat not commonly used by the host (Wyllie 1981, pers. obs.). **The post-fledging period of the cuckoo is by far the least studied stage that occurs in the breeding grounds** (Tyller *et al.* 2018). There are indications that fledgling cuckoos use vegetation that is not used either for host nesting or adult cuckoo activity, and this would have implications for suitability of habitat for cuckoo breeding. Further, standardised observation of fledgling cuckoos, such as transect surveys or telemetry, is required to establish whether habitat requirements are distinct at this stage.

BREEDING GROUND 2 – ADULT PREY AVAILABILITY

While the cuckoo is regarded as a habitat generalist, its ability to handle and consume large moth and butterfly (Lepidoptera) larvae, many of which have physical and chemical defences which deter most predators (Wyllie 1981), has led to suggestion that the cuckoo specialises on this prey. Anatomical studies have highlighted adaptations suited to consuming them as a key prey type. These include bill morphology that facilitates prey handling at the base of the bill, which may allow larger prey with unpalatable parts to be handled more easily and for extended periods (Korzun *et al.* 2003); and the ability to shed the stomach lining, possibly to eject larval spines and hairs after ingestion (McAtee

1906, 1917). However the importance of this prey taxon is presumed, from biased and often anecdotal methods of study in the historical literature, and the true composition of cuckoo prey is ultimately poorly understood. Determining whether the cuckoo has a specialised or broad base of prey, and their distribution, is essential to understanding the species' vulnerability to environmental change.

Cuckoo prey composition and evidence for preferred prey

Most knowledge of cuckoo prey taxa originates from field observations at distance (e.g. Crawshaw 1963, Bottomley & Bottomley 1975, Armitage 1978, Nakamura & Miyazawa 1997). Detection and identification by observers is more likely with prey items that are more visible and handled for longer periods before ingestion. Prey observed and identified is therefore biased in favour of prey taxa that are large, gregarious or have defences which require prolonged handling to disarm, and all three traits are frequent among the Lepidoptera larvae that dominate historic cuckoo prey data (Wyllie 1981). Study of the bill and jaw anatomy of common cuckoos and other Cuculidae shows adaptations that increase ability to handle prey at the bill base, and disarm toxic prey or subdue larger prey (Korzun *et al.* 2003). Cuckoos are able to shed their stomach lining (McAtee 1906) which *in vivo* has been sometimes found lined with irritant caterpillar hairs (McAtee 1917). These characteristics suggest cuckoos have adapted to feed predominantly on large defensive invertebrates in high volume. If cuckoos are specialising on large moths (Lepidoptera) there is cause for concern as many species in this taxon have declined drastically in western European countries such as Britain (Conrad *et al.* 2006, Fox *et al.* 2014). **Long-term change in adult moth numbers shows strong correlation with land use, and species historically recorded in the cuckoo diet** (Wyllie 1981) **have declined more significantly than the overall trend in British moths** (Denerley *et al.* 2018).

While studies of full-grown (adult and fully-developed juvenile) cuckoos' stomach contents have frequently recorded an abundance of Lepidoptera larvae, other specimens had consumed entirely beetles Coleoptera. Three studies in the literature inspected more than 15 specimens (Collinge 1925,

Dement'ev & Gladkov 1966, Ishizawa & Chiba 1966). A correspondent to Lowe (1943) noted taxa in high abundance in cuckoo stomachs were those likely to be most visible by contrasting coloration with substrate. This fits with observations that the cuckoo is clearly a visual predator, swooping from a raised vantage point to pick up prey from substrate (Wyllie 1981). High abundance of a low diversity of prey in stomach contents is likely to largely relate to the stomach contents studies' tendency to capture relatively few food intake periods, but to an extent also concurs with observations of cuckoo foraging on aggregations of prey that are temporarily abundant such as gregarious and tent-living caterpillars (Lepidoptera) (Wyllie 1981).

While a wide range of invertebrates other than Lepidoptera have been recorded as cuckoo prey, the underlying methodology or evidence are rarely stated, and there is no indication of frequency. This limits our ability to determine whether these are taken during standard foraging or are sought in the complete absence of their preferred prey e.g. late prey emergence relative to spring cuckoo arrival. Other reported prey taxa are adult flies (Diptera), beetles, grasshoppers (Orthoptera), sawfly larvae *Nematus sp.* (Hymenoptera), soil-surface larvae of beetles and flies including leather-jackets (Tipulidae) and wireworms (Elateridae), millipedes (Myriapoda) and molluscs (Gastropoda) (Abbey 1909 as reported in Smith 1930; Wyllie 1981). Cuckoo diet requires updated study which better captures the size range of prey consumed than distant field observation. Molecular analysis of faeces combines the certainty that prey present in faeces have been consumed by the bird, with the potential for finer taxonomic identification afforded by molecular-based methods (Pompanon *et al.* 2012, King *et al.* 2015).

Commuting and home-ranges, and implications for feeding and condition

Tracked individuals have demonstrated that cuckoos in a range of breeding habitats undertake journeys away from their breeding habitat to feed (Wyllie 1981, Droscher 1988, Nakamura & Miyazawa 1997, Vogl *et al.* 2004). The feeding habitat types used by tracked birds from different breeding habitats are summarised in Table 1. Daily 'commuting' significantly contributes to the overall scale of a cuckoo individual's home range, of 10^1 to 10^2 Ha, with breeding

behaviour concentrated in smaller ‘territories’ of much narrower habitat type (Table 2). In non-breeding areas cuckoos similarly move between feeding areas and appear to occupy a home range, which is smaller in area than home ranges held by the same individuals in the breeding grounds (Williams *et al.* 2016). Commuting between egg-laying/nesting area and a distinct foraging habitat is common to large invertebrate-feeding birds. Grey-headed woodpeckers *Picus canus* occupy similar-sized home ranges during breeding to cuckoos, and home ranges two orders of magnitude larger in winter (Rolstad & Rolstad 1995). Tracked European nightjars also feed in distant and contrasting areas of habitat (Evens *et al.* 2017). Common swifts range widely in relation to weather-fronts, which affect availability of prey (Koskimies 1947). In summary, while previous suggestions that breeding habitat alone holds insufficient prey for the cuckoo may be correct (Nakamura & Miyazawa 1997), the above studies suggest that ranging over relatively large areas of breeding habitat and other habitat is common among large insectivores. In the cuckoo and other brood parasites, ranging behaviour is made more possible by birds providing no parental care (Nakamura & Miyazawa 1997).

The diversity of feeding habitat types exploited by tracked birds from a given population (Table 1) suggests that cuckoos are generalist in feeding habitat. Home ranges of tracked individuals were noted to include ‘edge’ habitat where open and closed habitats met, in both breeding and non-breeding periods (Williams *et al.* 2016) so this may be a key overarching feature of preferred habitats. Favoured feeding habitats in Britain alone include, “hedges, woodland rides, patches of heather, areas of scrub, reedbeds, [and] disused railway lines,” (Wyllie 1981). Negative trends have been reported in the abundance and status of many of these habitat types, but our lack of full understanding of cuckoo diet makes it difficult to determine what role loss of feeding habitat could play in cuckoo decline. Foraging habitat of tracked nightjars, which similarly commute to feed, included habitats previously not associated with the species, such as cultivated grasslands (Evens *et al.* 2017). Recent telemetry studies of cuckoos suggested that arable land was generally not used for foraging (Vogl *et al.* 2004), though surveys of bird foraging in pastoral land indicated ground

Table 1. Feeding habitats used by tracked cuckoos from various breeding populations.

Study	Breeding habitat	Feeding habitat
Chance (1922, 1940), UK	Grassland surrounded by woodland	Feeding never recorded within grassland, woodland suspected
Wyllie (1981), UK	Reedbed	Railway scrub, orchard, reedbed, meadow with mature hedges
Dröscher (1988), Germany	Suburban patchy habitats	Woodland preference, distant from laying sites
	Rural habitats	Woodland preference, close to laying sites
Nakamura & Miyazawa (1997), Japan	Reedbed, riverine habitat with tall tree groves, rural and suburban human sites	Densely forested mountains (concentrated feeding). Human sites (suburban garden trees) (sprawled feeding) by birds breeding furthest from mountains. Some apparently suitable habitats e.g. orchards, rural human sites and rice fields were NOT used.
Vogl *et al.* (2004), Czech Republic	Pond edges, reed and herb vegetation	Stands of oak and pine trees

feeding by cuckoos (Romanowski & Zmihorski 2008). Local habitat composition may affect necessary commuting distance for cuckoos, and this has been shown to correlate negatively with total time spent in feeding areas per day (Nakamura & Miyazawa 1997). All tracked cuckoos in the study appeared to maximise timespent in their breeding area, but those commuting further and feeding for less time per day were expected to be in poorer body condition which may affect fecundity and productivity. If range size or commute distance are forcibly increased by reduction in suitable food supply or habitat, this may

Table 2: Range area sizes of individuals in common cuckoo breeding populations. Home = overall range, all behaviours. Female breeding = area over which females laid eggs. Male breeding = area over which males interacted with females. Male song = area over which males perched and gave breeding call.

Study	Host species	Range area (Ha)	Method
Wyllie 1981, UK	*Acrocephalus scirpaceus*	Male home: 1256 Female home: 1256	Circular area calculated from estimated range diameter of 4 km
Nakamura & Miyazawa 1997, Japan	*Acrocephalus arundinaceus*, *Cyanopica cyanus*, *Lanius bucephalus* (combined sample)	Male home: 302 ± S.E. 28 (n=11) Female home: 326 ± 40 (n=16) Male song: 40.9 ± 4.9 (n=19) Male breeding: 67.5 ± 8.9 (n=21) Female breeding: 63.6 ± 8.5 (n=22)	Radio telemetry
	A. arundinaceus	*Female breeding: 51.2 ± 6.2 (n=11)*	Radio telemetry
	C. cyanus	*Female breeding: 75.1 ± 17.4 (n=8)*	Radio telemetry
	L. bucephalus	*Female breeding: 80.4 ± 45.0 (n=2)*	Radio telemetry
Vogl *et al.* 2004, Czech Republic	*Acrocephalus scirpaceus*, *A. schoenobaenus*, *A. arundinaceus*,	Female home: median = 60.8, q_1 = 40.88, q_3 =	Radio telemetry

	A. palustris (combined sample)	115.1) (n=7) Female breeding: median = 4.8, q_1 = 3.6, q_3 = 8.1) (n=7)

S.E. = standard error, q1 and q3 = quartiles 1 and 3

constitute a potential land-use threat to cuckoo populations mediated through prey availability. Anthropogenic factors influencing avian foraging distances have previously been indicated in breeding seabirds (Bertrand *et al.* 2012).

The threat of reduced prey availability due to advancing spring prey emergence

Cuckoos spend around three weeks after arrival at the breeding grounds before egg laying is recorded (Lack 1968, Wyllie 1981), and staging whilst migrating north to the breeding grounds in spring is limited north of western Africa (Willemoes *et al.* 2014, Hewson *et al.* 2016). Cuckoos are therefore indicated to adopt an 'income' breeding strategy. This strategy entails dedicating a significant time budget to feeding on food sources present on the breeding grounds to restore and maintain their body condition over the breeding period, compared to 'capital' breeders such as Arctic-breeding wildfowl which use the body condition resultant from feeding earlier in the annual cycle to sustain them through the breeding period (Drent & Daan 1980). On this basis, **breeding ground food sources are likely to be of additional importance to cuckoos achieving condition to breed.** Given that cuckoo prey is invertebrate with a suggested predominance of Lepidoptera, and that arrival of long-distance migrants is less likely to track earlier spring temperature rises and emergence of lepidopteran larvae (caterpillar) prey (Both & Visser 2001), it is urgent to determine whether cuckoo prey caterpillar species are shifting their timing of spring emergence, and what other taxa are available for cuckoos if their arrival is too late or premature. Nakamura and Miyazawa (1997) noted anecdotally that cuckoo spring arrival in the study area in Japan occurred later than deciduous

tree and grass foliation, or emergence of caterpillars. While arrival dates of cuckoo are well documented (Davies 2015), phenology data for known cuckoo prey taxa is limited to historic data on first encounter dates of adult moths. Analysis of this data against weather data suggests complex responses of lepidopteran adult phenology to spring or autumn climate (Sparks *et al.* 2006). Lepidopteran larval phenology may significantly relate to that of adults, but no studies of this relationship are known from the literature. While growth and peaks in biomass of caterpillars hatching from eggs in woodland canopy can be monitored indirectly using 'frass' nets (Tinbergen & Dietz 1994), larvae recorded as cuckoo prey predominantly overwinter as large larvae and feed near or on the ground (Wyllie 1981, Waring & Townsend 2017), and so cannot be monitored with frass nets. It may be possible to carry out standardised surveys for presence of large larvae of known cuckoo prey such as drinker moth *Euthrix potatoria*, which are relatively conspicuous in open semi-natural habitats. These surveys could be used annually to estimate date of first detection at a given site.

In summary, while major studies into phenology of bird breeding and lepidopteran emergence (e.g. Both & Visser 2001) focus on larvae emerging en masse from eggs, that are important as a food source for new nestlings, the spring emergence of cuckoo prey is predominantly large larvae that have overwintered (and may emerge gradually), and are important as a food source for adults. However, cuckoos are indicated to have an 'income' breeding strategy where body condition is restored at the breeding grounds, therefore loss of synchrony with caterpillar phenology could have a significant impact on cuckoo breeding success. Studies of the adult cuckoo diet, and the phenology of adult cuckoo arrival from migration (from existing data e.g. Sparks *et al.* 1999) and prey caterpillar emergence (from new field data and searching field records of caterpillar sightings, or using adult flight season phenology data e.g. Sparks *et al.* 2006) are urgently needed to determine whether cuckoos are arriving early or late relative to presence of caterpillar prey, and what other invertebrates may be consumed by cuckoos if caterpillar emergence is delayed.

Reduced prey availability as a potential ecological factor through which cuckoos have declined in agricultural habitats

Loss of food resource may explain why cuckoo numbers have declined and not recovered in agricultural habitats, despite thriving populations in the most common farmland cuckoo host species (Fig. 2). The trends of individual species of farmland bird are variable, as are their proposed most likely mechanisms of decline (Newton 2004b), but farmland birds are one of the most sharply declining groups collectively (Hayhow *et al.* 2017). As a species classified as generalist, the cuckoo is not included in the farmland bird population index (e.g. Hayhow *et al.* 2014), but potentially specialist sub-populations target farmland host species such as dunnock (Wyllie 1981). Occurrence of cuckoo eggs or young in the nests of dunnock (as well as other farmland hosts European robin, and pied wagtail *M. alba yarrellii*) decreased according to national Nest Record Scheme (NRS) data (Brooke & Davies 1987). This analysis detected a simultaneous increase in cuckoo occurrence in reed warbler nests. This host remains commonly used in Britain (Lindholm 1999, Davies 2015) and has increased in agricultural habitats (Massimino *et al.* 2017). Combined CBC and BBS trends for robin and pied wagtail show long term stability, while trends for dunnock show a period of decline from 1975 to 1985 followed by recovery. From 1995 to 2011, abundance trends of farmland hosts were non-significant or showed significant increases, but cuckoo numbers on farmland in the UK significantly declined (Fig. 2, Massimino *et al.* 2017). Agricultural areas that retained their cuckoo population commonly contained suitable wetland habitat for reed warbler nesting (Denerley 2014). This suggests that resources other than host abundance, such as adult food supply, may have driven declines in lowland agricultural habitats.

Threat of intensive agricultural practice

A key cause of large-scale environmental change capable of reducing resource availability for land-birds is land-use change. Anthropogenic trends in management of landscapes for food production and development may result in loss of biodiversity or habitat structure required for bird foraging and breeding, including at national or international scales. In lowland Britain, agricultural land

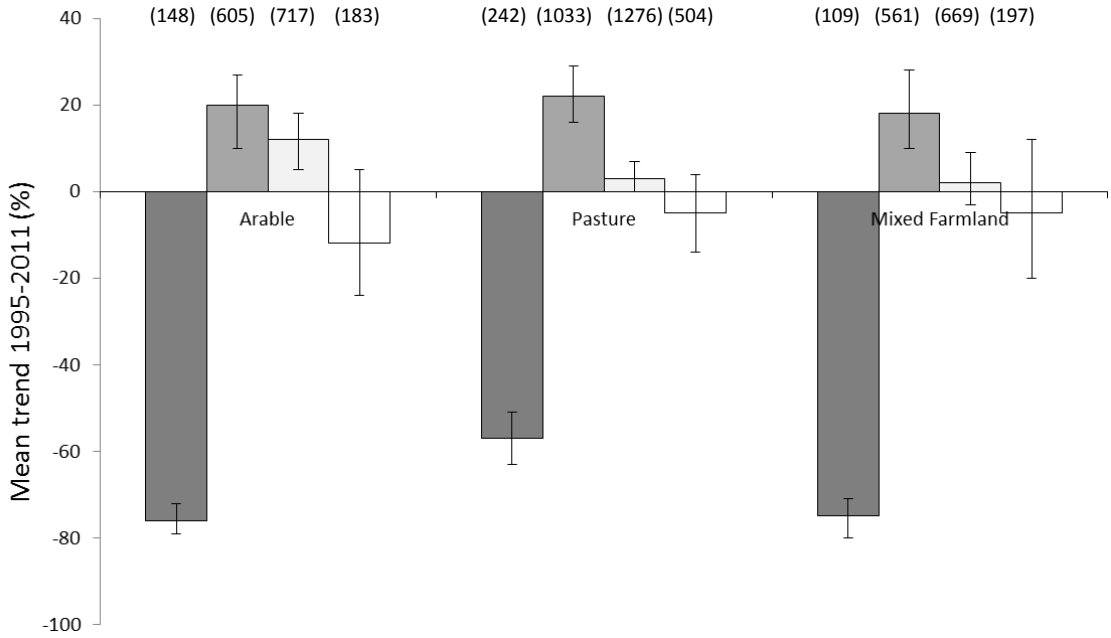


Figure 2. Mean trend 1995-2011 in numbers of cuckoo (dark grey bars) and host species dunnock (mid grey), robin (pale grey), and pied wagtail (white) on transect surveys of arable, pastoral and mixed farmland within the BTO Breeding Bird Survey (BBS). Error bars show 95% confidence intervals and numbers in parentheses are mean annual sample sizes. Data originally published in Massimino *et al.* 2017).

traditionally provided an important form of open lowland habitat, supportive of biodiversity through production of multiple crop types (often both plant and animal-based) within single farms (Robinson *et al.* 2001). More recently, increased demand for food production and financial incentive from the Common Agricultural Policy have encouraged large-scale intensification of lowland cultivation, towards less diverse and higher-yield crops (Donald *et al.* 2001), or sole focus on animal husbandry. This has replaced mixed farming by polarisation to intensive arable or intensive livestock agriculture (Robinson *et al.* 2001). The associated conversion, mechanisation and application of chemicals have been linked to loss of farmland biodiversity (Stoate *et al.* 2001, Robinson and Sutherland 2002). For both Britain and Europe, birds of farmland have as a group declined significantly more than the overall bird fauna, and more than any other population indexed bird assemblage e.g. woodland or wetland birds (Hayhow *et al.* 2014).

There are numerous aspects of intensive lowland agricultural land use that stand to impact, or have already impacted, on cuckoos and their hosts. Nesting habitat of cuckoo hosts in open habitats in Europe includes complex ground vegetation (pipits, wagtails), traditional field boundary vegetation (dunnock, robin, red-backed shrike *Lanius collurio*) and vegetation in ditches and wet areas (*Sylvia* and *Acrocephalus* warblers). The drive during intensification to maximise the cropped area within fields, and use fields in successive years, has increased likelihood of ground nesting vegetation being permanently removed from farmed land. The trend towards increasing field sizes for machinery access means intensification often involves removal of traditional field boundaries, and pressures to more efficiently maintain boundaries have brought about use of mechanical flailing and severe cutting of hedgerows, limiting their capacity for nesting birds. Boundary ditches are likely to be removed to aid machinery access. Anthropogenic maintenance of more stable, favourable water levels for agriculture and development through drainage infrastructure is likely to limit extent of wetland areas in farmable land. Water management has previously been associated with declines of great reed warblers in the Netherlands (Graveland 1998), however common reed warblers *A. scirpaceus* appear to nest readily in drier and drained habitat provided grazing does not reduce reed vegetation (Williams *et al.* 1983). In summary, **cuckoo host nesting habitats are typically regarded as counterproductive features to intensive lowland agricultural production**, and this is likely to explain the loss of meadow pipits from much of Britain's lowland grassland, and continued declines where they remain in these habitats (Massimino *et al.* 2017). As indicated above, habitat and host specialism of individual cuckoos means removal of specific habitats may eliminate all suitable breeding habitat for local cuckoo populations. In a lowland example, removal of rough grasslands and preservation of reedbeds could potentially eliminate all local cuckoo breeding habitat if all local cuckoos target grassland host nests. But further to this, **foraging habitat of cuckoos is characterised by presence of hedges or trees** (Table 1) **which are similarly likely to be removed in intensive lowland agriculture** to increase efficiency and lower requirement for boundary and non-crop maintenance. The application of pesticides associated with intensive land use directly aims to reduce abundance of crop pest invertebrates, which includes prey of cuckoos, host

species and their nestlings, and may indirectly affect hosts by reducing the food source of their prey. This latter mechanism is also the case with herbicides and the general removal of non-crop plants. Plant diversity has also declined through high phosphorus input, intensive grazing and being outcompeted by planted forage grasses for livestock (Vickery *et al.* 2001). Some invertebrate taxa of open habitats have life stages vulnerable to high mortality from agricultural practices such as ploughing. These include species with egg and early instar stages in soil, such as Lepidoptera, Orthoptera, and sawflies (within Hymenoptera). Mechanised mowing and cutting during the bird breeding season is destructive of foliar arthropods. Fertilizer applications to soil are known to alter the assemblage of invertebrates, with many taxa intolerant of high application rates (Vickery *et al.* 2001). Overall **intensive land management practices are likely to entail reduction in invertebrate prey availability, as well as removal of host nesting habitat, and cuckoo and host foraging habitat.**

Demand for agricultural food production is projected to increase by 100% between 2000 and 2050 (Tilman *et al.* 2002). The discussion for reconciling food production with preserving biodiversity in lowlands has fallen predominantly into the dichotomy of 'land sparing', or 'land sharing' also referred to as 'wildlife-friendly farming' (reviewed Green *et al.* 2005, Grau *et al.* 2013). Land sparing is representative of current conservation and agricultural policy in Europe, wherein areas allocated for nature preservation are distinguished from areas of land where agricultural production is carried out (Grau *et al.* 2013). Under this system, increasing demand is met through efforts to increase yields in existing agricultural land. This minimises requirement for new conversion of natural land to farmland (Green *et al.* 2005). Land sharing proposes biodiversity conservation effort should be exercised across all land within an area, including land used for food production. Resulting yield is a compromise with efforts to promote biodiversity, but is proposed to be maintained or even increased with correct planning and management (Pywell *et al.* 2015). The scale across which both land sparing and land sharing are discussed and modelled is variable (Egan & Mortensen 2012). 'Rewilding' is a yet more ambitious policy geared towards restoration of natural processes, but

is limited in empirical research and is broadly plastic in its definition (reviewed Lorimer *et al.* 2015). Experiments in active re-wilding (with management such as species re-introduction and habitat restoration) are few, and set few to no goals regarding food production within their landscapes (Lorimer *et al.* 2015).

Virtually all areas in Britain where cuckoo abundance was stable or increased between the two most recent BTO atlases (Balmer *et al.* 2013) are upland areas that carry large-scale designations such as National Park or Area of Outstanding Natural Beauty (AONB). These are variously designated for their rural aesthetic, wildlife and cultural heritage, as opposed to conservation. Retention of cuckoos in these areas of agriculture (predominantly sheep livestock) and semi-natural habitats suggest upland 'land sparing' at the scale of national parks (often 10^2 to 10^3 km^2) is suitable for cuckoos. (Cuckoo populations have been studied across such landscapes in Czech Republic (Vogl *et al.* 2002) and Japan (Nakamura & Miyazawa 1997) but the regional population trend in these study areas is not reported.) In comparison, cuckoos have declined in lowlands in Britain where arable and intensive pastoral agriculture may exist continuously over similar area sizes to national parks. In Britain and Europe, cuckoo populations are increasing in Scotland (Massimino *et al.* 2017, Harris *et al.* 2018) and Finland (Laaksonen & Lehikoinen 2013) where semi-natural or wild land predominates continuously over areas similar to the above. Cuckoos have therefore increased in extensive areas with lowest-intensity management intervention.

Conclusions

Results from tracking studies have attracted wide public attention to the migration and conservation status of the common cuckoo in Britain, and across Europe and Asia (Hewson *et al.* 2016, Birding Beijing 2018). Studies of the non-breeding period have also highlighted that European cuckoos are taking two different southbound migration routes, spend the most southerly period of their migration cycle in southern Africa, and stage in western Africa early in the Northern Hemisphere spring before returning to Europe. Tracking studies are developing to use additional remotely-sensed data, and have shown that cuckoo movements in the non-breeding period closely track seasonal booms in

primary production and associated invertebrate availability (Jacobsen *et al.* 2017, Thorup *et al.* 2017). These show the role remote studies can play in addressing questions of why long-distance migrant birds are collectively undergoing breeding declines. Ultimately, however, addressing these questions will require *in situ* study of the non-breeding periods of cuckoos and other long-distance migrants.

Climatic change effects have been a major feature of breeding ground research of the common cuckoo in recent years. While studies have successfully made use of long-term datasets to confirm the cuckoo has advanced its phenology less than hosts that overwinter as residents or short-distance migrants, there is uncertainty in the effect of this climate-mediated shift on cuckoo populations. Evidence has meanwhile mounted that female cuckoos have specialist laying habitat. It is reasonable at this stage to proceed on the basis that loss of such habitat could render females devoid of laying habitat and unable to successfully breed if similar habitat cannot be found within the home range. On the basis of genetic studies, males also have a preferred 'primary' host and may concentrate breeding effort in a narrow habitat range containing that host, in which case males would be as vulnerable as females above. Work is urgently needed to confirm by independent means whether cuckoos mate assortatively, as this has considerable implications for population viability. Combined with host specificity, evidence for variation in cuckoo fledging mass between host species suggests juvenile cuckoo survival may vary between populations using different hosts and habitats.

Land use change and intensification involves practices likely to have negatively impacted on populations of the two main hosts in Britain and of adult cuckoo prey, which at present is understood to comprise mainly large moth caterpillars. Cuckoos are likely to be income rather than capital breeders, in which case breeding ground food sources are of immediate importance to cuckoos entering condition to breed. While the similarity of cuckoo home ranging behaviour to other large insectivores suggests commuting several kilometres to feed is not a symptom of habitat fragmentation, there are valid concerns that land conversion may create greater extents of unsuitable habitat within cuckoo home ranges. Land use practices that remove suitable nesting habitat for host species, or

remove tall structures such as trees, respectively, reduce the host nests resource available to cuckoos, or the perches required for cuckoo females to monitor nests and parasitise them. In England, stronghold cuckoo populations show spatial association with extensive areas of semi-natural habitat, often as large-scale areas of land sparing. However the effects of the touted alternatives of land-sharing, wildlife friendly farming and rewilding on cuckoos is not known.

iii) What are the priority research areas regarding the common cuckoo?

Study throughout the annual cycle of migration and other life events will be required to understand the causes of survival, mortality and productivity trends and variation in the cuckoo. This is likely to be achievable only through identification and prioritisation of key regions, and co-ordination and collaboration among conservation bodies along the migration route. *In situ* study of the non-breeding ecology of the cuckoo in Europe and Africa should be initiated in the immediate future, as this could support the remote-sensing approaches to migrant bird study that are being applied presently. Specific study is required regarding what cues may be used by cuckoos for their departure from northbound staging areas in west Africa in early spring. Study is required concerning abundance, demography (survival rates, age and sex ratios), body condition and ecology, throughout the migration cycle. The most immediate of these are annual measurements of abundance and body condition of cuckoos at sites where these have previously been measured. Comparisons of mean and inter-annual variance in these measurements between distant time periods would give first indications of cuckoo status at these locations. Body condition on arrival at the breeding grounds has received little attention, but analysis of long term data from ringing and collection of new data are potentially invaluable, especially if combined with data on weather conditions in key regions of the migratory cycle.

To aid future analyses of climatic and other environmental effects on cuckoo populations, governmental, public and volunteer interest must be maintained in funding and contributing to long term monitoring of birds, their nests and environmental variables. Success in isolating effects on populations of cuckoos

of a specific ‘host-race’ or *gens*, may lie in nest record scheme datasets, though the non-random nature of such datasets makes analysis particularly challenging. Stepping back from this, updates detailing the recent host composition of the cuckoo in Britain (and many other countries) have not been carried out in decades (e.g. Brooke & Davies 1987 most recently for Britain) but would be invaluable for tracking shifts in host use of national populations. Information on the diet of the cuckoo is also dated, but requires application of novel molecular based methods that may overcome some of the biases of field observation and represent less of a snapshot than stomach contents studies. Between host systems studied intensively at the nest so far, cuckoo nestling diet has been shown to have both similarities and differences to that of host broods, and work of this type is required in further host species and more populations of each host. Crucially to future studies of this type, effort should be made to also quantify survival rate of host broods and of cuckoo young, or the population trajectory of cuckoos local to the study, as an indication of how study populations relate to their host or habitat-specific ecology.

Research of the post-fledging period of the cuckoo is particularly scarce and is a priority area of research on the breeding ground for this reason. A significant amount of data on habitat use, change in mass, time to independence and prey composition is likely to be obtainable through field observation and radio telemetry. However to establish the breeding habitat and host, and degree of dispersal, of juvenile cuckoos, first-year birds will require tracking using PTT tags on sufficient number of birds as to observe several migrations back to the breeding region. There is a difficulty thus far in that juveniles typically weigh less than adult birds and are less suited to carry even the most lightweight PTT tags available. However fledgling mass varies with study species, with nestlings raised by great reed warblers cited to gain the most mass before fledging (Kleven *et al.* 1999). PTT or PIT (passive integrated transponder) tag technology of sufficient spatial accuracy could also be used on the breeding grounds, for quantifying and comparing clutch sizes of female cuckoos (total number of eggs laid), and studying to what extent cuckoos mate with others of matching and mismatching natal host or host specialism. One other application of PTT tagging to cuckoo demography would require further reductions in tag

mass. Ability to track fledglings to future breeding seasons (with negligible variation in tag effect across the normal juvenile body mass range) will be required to determine survival rates of juveniles raised by different host species. Similar to existing work by Kleven *et al.* (1999), this will require cross-fostering to remove confounding effects of higher quality cuckoos using higher quality hosts. These questions all have important conservation biology relevance, but can largely not be addressed until tag technology is further miniaturised.

Based on the above body of research, cuckoos have from been revealed to show exceptional variation between populations and between habitats; in host species (and related to this, nest habitat, cuckoo egg-laying date, nestling diet, nestling survival and weight at fledging), breeding habitat, foraging habitat, home range size, and population trend. The generalist description of this species conceals individual and sub-population specialism. On this basis, studies of cuckoos should wherever possible document the conservation status or trend trajectory of specific study populations. This will assist in establishing what habitats, host species and resources are associated with declining and non-declining populations.

Present study objectives

This review has highlighted knowledge gaps relating to all parts of the cuckoo's annual cycle, and demonstrated that cuckoo populations may be impacted by a range of factors, including high mortality stages of migration, climatic impacts on phenology of vegetation and invertebrates throughout the migration route, and hosts on the breeding grounds, and limits on population viability and mate availability due to host and habitat specialism. However, the present study is focused on breeding ground habitat, host and food resources of the cuckoo, and the potential impact of land use change on these resources.

In chapter 3 I use field data from a study area on Dartmoor, an upland area of Devon, UK, to test hypotheses that meadow pipits, a major host of common cuckoo parasitism in Britain, display selection of foraging habitat that has structyral heterogeneity, and that meadow pipit nestling body condition and

nestling provisioning by adult pipits vary significantly with fine-scale breeding and foraging habitat respectively. While this chapter predominantly repeats past studies of meadow pipits breeding in other study habitats, crucially it provides a basis to understanding the habitat associations of the host in the present study habitat and area. In chapter 4, similar hypotheses are then tested for a wider sample of meadow pipit nests containing either non-parasitised broods of pipits or a single cuckoo nestling and no pipit nestlings, to determine whether adults at nests parasitised by the cuckoo show significant differences in nestling-provisioning behaviour, including visit rate, prey load size, foraging distance and habitat use. This addresses a key knowledge gap in research of host provisioning of cuckoos compared to host nestlings, and has significance to inferring whether hosts expend greater energy raising a cuckoo than a host brood in the same habitat or study area. This in turn provides indication of whether raising a cuckoo is more difficult for a host pair under equal conditions or resource availability. Chapter 4 also uses molecular analysis of prey remains in faeces of cuckoo and meadow pipit nestlings and fledglings to determine prey composition in cuckoo and host nestlings. This is the first comparison of cuckoo and host nestling diet in the meadow pipit host. Molecular analysis of prey remains is used in chapter 5 on faecal samples from adult cuckoos ranging across the same study area, to test the hypothesis that adult cuckoos feed predominantly on large Lepidoptera larvae but also species of other invertebrate taxa that may be small or soft-bodied and less readily detected by field observational studies. The present study represents the first reported application of molecular analysis of prey from faeces to the common cuckoo. In chapter 5 I also test the hypothesis that larval lepidopteran prey of the cuckoo in Britain, as identified from a national survey of photographic captures of predation, are similar to the prey assemblage previously reported in the autecological review by Wyllie (1981). The two adult diet analyses in this chapter aim to update and improve the detection and identification of cuckoo prey. In chapter 6, rates of occurrence of cuckoo prey moths documented in Wyllie's (1981) review are examined within a historical moth trapping dataset for the county of Devon to test the hypothesis that annual trend in rate of occurrence of some or all cuckoo prey moths is more positive in upland areas with extensive semi-natural habitat, over a 13 year period. This focused,

regional study follows recent national aggregated analyses suggesting that habitat-specific trends in abundance of moths documented as cuckoo prey by Wyllie (1981) are similar to habitat-specific trends in adult cuckoo abundance. In chapter 7, I synthesise the findings of the previous chapters' analyses, with regard to how land use intensification historically and currently impacts on aspects of the cuckoo breeding ground resources of habitat, hosts and prey highlighted by the present study.

References

Abbey, G. (1909) *The Balance of Nature and Modern Conditions of Cultivation*. London: G. Routledge & Sons.

Åkesson, S., Klaassen, R., Holmgren, J., Fox, J. W., and Hedenström, A. (2012) Migration routes and strategies in a highly aerial migrant, the common swift *Apus apus*, revealed by light-level geolocators. *PLoS One* 7(7).

Alverez F. (1993) Proximity of trees facilitates parasitism by Cuckoos *Cuculus canorus* on Rufous Warblers *Cercotrichas galactotes*. *Ibis* 135: 331–331.

Araújo, M. S., Bolnick, D. I., and Layman, C. A. (2011) The ecological causes of individual specialisation. *Ecology Letters* 14(9): 948–958.

Armitage, J. S. (1978) 'Feeding methods and prey of the cuckoo', *British Birds*, 71, pp. 590–590.

Atkinson, P.W., Adams, W.M., Brouwer, J., Buchanan, G., Cheke, R.A., Cresswell, W., Hewson, C.M., Hulme, M.F., Manvell, A., Sheehan, D.K. and Small, R.D. (2014). Defining the key wintering habitats in the Sahel for declining African-Eurasian migrants using expert assessment. *Bird Conservation International*, *24*(4): 477-491.

Auniņš, A. (2018) Declines in breeding birds continue: the most affected are the species wintering in Africa and farmland birds. (in Latvian). *Putni dabā* 81: 10–15.

Bairlein, F., Norris, D. R., Nagel, R., Bulte, M., Voigt, C. C., Fox, J. W., Hussell, D. J. and Schmaljohann, H. (2012) Cross-hemisphere migration of a 25 g songbird. *Biology letters*. doi:p.rsbl20111223.

Baldamus, E. (1892) *Das Leben der europa¨ischen Kuckucke*. Berlin: Parey.

Balmer, D., Gillings, S., Caffrey, B., Swann, B., Downie, I., and Fuller, F. (2013) *Bird Atlas 2007–11.* Thetford: BTO.

Bán, M., Moskát, C., Fülöp, A., and Hauber, M. E. (2018) Return migration of Common Cuckoos (*Cuculus canorus*) between breeding grounds in Hungary and wintering grounds in Africa as documented by non-PTT GPS technology. *Journal of Ornithology* 159(2): 337–344.

Barrett, R. T. (2014) Has climate change resulted in a mismatch between the spring arrival of the Common Cuckoo *Cuculus canorus* and its hosts in North Norway? *Munin.Uit.No*. Accessed: 9th February 2019 <https://munin.uit.no/bitstream/handle/10037/7116/article.pdf?sequence=3. >.

Bearhop, S., Hilton, G.M., Votier, S.C. and Waldron, S., (2004). Stable isotope ratios indicate that body condition in migrating passerines is influenced by winter habitat. *Proceedings of the Royal Society of London B: Biological Sciences*, *271*(Suppl 4), pp.S215-S218.

Beresford, A. E., Sanderson, F. J., Donald, P. F., Burfield, I. J., Butler, A., Vickery, J. A., and Buchanan, G. M. (2018) Phenology and climate change in Africa and the decline of Afro-Palearctic migratory bird populations. *Remote Sensing in Ecology and Conservation*. doi:10.1002/rse2.89.

Berthold, P. (1996) *Control of bird migration.* Springer Science & Business Media.

Bertrand, S., Joo, R., Smet, C. A., Tremblay, Y., Barbraud, C., and Weimerskirch, H. (2012). Local depletion by a fishery can affect seabird foraging. *Journal of Applied Ecology*, *49*(5), 1168-1177.

Birding Beijing (2018) The Beijing Cuckoo Project. *Birding Beijing*. Accessed: <https://birdingbeijing.com/beijing-cuckoo-project/. >.

Birdlife International (2016) *Cuculus canorus*, *The IUCN Red List of Threatened Species 2016: e.T22683873A86119034*. Available at: http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22683873A86119034.en (Accessed: 8 February 2019).

Blackburn, E., and Cresswell, W. (2016) High within-winter and annual survival rates in a declining Afro-Palaearctic migratory bird suggest that wintering conditions do not limit populations. *Ibis* 158(1): 92–105.

Boele, A., van Bruggen, J., Slaterus, R., Vergeer, J.-W., and van der Meij, T. (2018) *Broedvogels in Nederland in 2016*. Nijmegen.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., and Forister, M. L. (2002) The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist* 161(1): 1–28.

Booker, H., Buckley, P., Alsbury, S., Walters, J., Exley, P., and Baldock, N. (2017) *East Dartmoor Moorland Breeding Bird Survey 2016.* Exeter.

Both, C. (2010) Food availability, mistiming, and climatic change. In Moller, A. P., Fiedle, W., and Berthold, P. (Eds.), *Effects of Climate Change on Birds*. Oxford: Oxford University Press.

Both, C., and Visser, M. E. (2001) Adjustment to climate change is constrained by arrival date in a long-distance migrant bird. *Letters to Nature* 411(May): 296–298.

Bottomley, J. B., and Bottomley, S. (1975) Cuckoos photographed feeding on magpie moth caterpillars. *British Birds* 68(12): 514–514.

Brooke, M. D. L., and Davies, N. B. (1987) Recent changes in host usage by cuckoos *Cuculus canorus* in Britain. *Journal of Animal Ecology* 56(3): 873–883.

Brooke, M. D. L., and Davies, N. B. (1989) Provisioning of nestling cuckoos *Cuculus canorus* by reed warbler *Acrocephalus scirpaceus* hosts. *Ibis* 131(2): 250–256.

Bussmann J (1947) Wachstum und Jugendzeit eines Kuckucks. *Ornithol Beob* 44: 41–49

Campobello, D., and Sealy, S. G. (2009) Avian brood parasitism in a Mediterranean region: hosts and habitat preferences of Common Cuckoos *Cuculus canorus*. *Bird Study* 56(3): 389–400.

Chance, E. P. (1922). *The cuckoo's secret*. Sidgwick and Jackson, Limited.

Chance, E. P. (1940) *The truth about the cuckoo*. London: Country Life.

Chodkiewicz, T., Meissner, W., Chylarecki, P., Neubauer, G., Sikora, A., Pietrasz, K., Cenian, Z., Betleja, J., Kajtoch, Ł., Lenkiewicz, W., Ławicki, Ł., Rohde, Z., Rubacha, S., Smyk, B., Wieloch, M., Wylegała, P., Zielińska, M., and P., Z. (2016) Monitoring Ptaków Polski w latach 2015–2016. *Biuletyn Monitoringu Przyrody* 15: 1–86.

Clarke, A. L., Øien, I. J., Honza, M., Moksnes, A., and Røskaft, E. (2001) Factors Affecting Reed Warbler Risk of Brood Parasitism by the Common Cuckoo. *The Auk* 118(2): 534–538.

Collinge, W. E. (1925) *The food of some British Wild Birds*. York: Yorkshire Herald Newspaper Company Ltd.

Conrad, K. F., Warren, M. S., Fox, R., Parsons, M. S., and Woiwod, I. P. (2006) Rapid declines of common, widespread British moths provide evidence of an insect biodiversity crisis. *Biological Conservation* 132(3): 279–291.

Cramp, S. (1988) *Handbook of the birds of Europe, the Middle East and North Africa: the birds of the western Palearctic.* Oxford University Press.

Crawshaw, K. R. (1963) Juvenile Cuckoo's method of feeding upon Cinnabar Moth caterpillars. *British Birds* 56: 28–28.

Cresswell, B., and Edwards, D. (2013) Geolocators reveal wintering areas of European Nightjar (*Caprimulgus europaeus*). *Bird Study* 60: 77–86.

Davies, N. (2015) *Cuckoo: cheating by nature*. Bloomsbury Publishing.

Dement'ev, G. P., and Gladkov, N. A. (1966) *Birds of the Soviet Union, Vol. 1*. Jerusalem: Israel Programme for Scientific Translations.

Denerley, C. (2014) *The impact of land use change on a brood parasite system: cuckoos, their hosts and prey*. University of Aberdeen.

Denerley, C., Redpath, S. M., van der Wal, R., Newson, S. E., Chapman, J. W., and Wilson, J. D. (2018) Breeding ground correlates of the distribution and decline of the Common Cuckoo *Cuculus canorus* at two spatial scales. *Ibis*. doi:10.1111/ibi.12612.

Donald, P. F., Green, R. E., and Heath, M. F. (2001). Agricultural intensification and the collapse of Europe's farmland bird populations. *Proceedings of the Royal Society of London B: Biological Sciences*, *268*(1462): 25-29.

Douglas, D. J. T., Newson, S. E., Leech, D. I., Noble, D. G., and Robinson, R. a. (2010) How important are climate-induced changes in host availability for population processes in an obligate brood parasite, the European cuckoo? *Oikos* 119(11): 1834–1840.

Drent, R. H., and Daan, S. (1980). The prudent parent: energetic adjustments in avian breeding. *Ardea* 68: 225-252.

Droscher, L. (1988) A study on radio-tracking of the European cuckoo (*Cuculus canorus canorus*). *Proc, Int. 100. DO-G Meeting, Current Topis Avian Biol., Bonn* 1988: 187–193.

Ducatez, S. (2014) Brood parasitism: A good strategy in our changing world? *Proceedings of the Royal Society B: Biological Sciences* 281(1780): 20132404.

Eaton, M., Aebischer, N., Brown, A., Hearn, R., Lock, L., Musgrove, A., Noble, D., Stroud, D., and Gregory, R. (2015) Birds of Conservation Concern 4: The population status of birds in the UK, Channel Islands and Isle of Man. *British Birds* 108(12): 708–746.

Egan, J.F. and Mortensen, D.A., (2012). A comparison of land-sharing and land-sparing strategies for plant richness conservation in agricultural landscapes. *Ecological applications*, *22*(2), pp.459-471.

Evens, R., Beenaerts, N., Witters, N., and Artois, T. (2017) Study on the foraging behaviour of the European nightjar *Caprimulgus europaeus* reveals the

need for a change in conservation strategy in Belgium. *Journal of Avian Biology* 48(9): 1238–1245.

Fossøy, F., Antonov, A., Moksnes, A., Røskaft, E., Vikan, J. R., Møller, A. P., Shykoff, J. a, and Stokke, B. G. (2011) Genetic differentiation among sympatric cuckoo host races: males matter. *Proceedings of the Royal Society B: Biological Sciences* 278(1712): 1639–1645.

Fuisz, T. I., and de Kort, S. R. (2007) Habitat-dependent call divergence in the common cuckoo: is it a potential signal for assortative mating? *Proceedings of the Royal Society B: Biological Sciences* 274(1622): 2093–2097.

Geary, S. (2000) *Dartmoor moorland breeding bird survey 2000.* Parke, Bovey Tracy.

Gibbons, D.W., Reid, J.B. and Chapman, R.A. (1993) *The New Atlas of Breeding Birds in Britain and Ireland: 1988 –1991*. T. and A.D. Poyser, London, UK.

Glue, D., and Morgan, R. (1972) Cuckoo Hosts in British Habitats. *Bird Study* 19(4): 187–192.

Grau, R., Kuemmerle, T. and Macchi, L., (2013). Beyond 'land sparing versus land sharing': environmental heterogeneity, globalization and the balance between agricultural production and nature conservation. *Current Opinion in Environmental Sustainability* 5(5): 477-483.

Graveland, J. (1998). Reed die-back, water level management and the decline of the Great Reed Warbler *Acrocephalus arundinaceus* in The Netherlands. *Ardea* 86: 187-201.

Green, M., Haas, F., and Lindström, Å. (2018) *Övervakning av fåglarnas populationsutveckling. Årsrapport för 2017– Rapport*.

Green, R. E. (1995) Diagnosing causes of bird population declines. *Ibis* 137(S1): S47-S55.

Green, R.E., Cornell, S.J., Scharlemann, J.P. and Balmford, A., (2005). Farming and the fate of wild nature. *Science* 307(5709): 550-555.

Grim, T. (2006) An exceptionally high diversity of hoverflies (Syrphidae) in the food of the reed warbler (*Acrocephalus scirpaceus*). *Biologia* 61: 235–239.

Grim, T. and Honza, M. (2001) Does supernormal stimulus influence parental behaviour of the cuckoo's host? *Behavioral Ecology and Sociobiology* 49(4): 322–329.

Grim, T., Tyller, Z., and Samaš, P. (2017) Unusual diet of brood parasitic nestlings and its fitness consequences. *The Auk* 134(3): 732–750.

Harris, S. J., Massimino, D., Gillings, S., Eaton, M. A., Noble, D. G., Balmer, D. E., Procter, D., and Pearce-Higgins, J.W. Woodcock, P. (2018) *The Breeding Bird Survey 2017. BTO Research Report 706*. Thetford.

Hayhow, D. B., Conway, G., Eaton, M. A., Grice, P. V., Hall, C., Holt, C. A., Kuepfer, A., Noble, D. G., Oppel, S., Risely, K., Stringer, C., Stroud, D. A., Wilkinson, N., and Wotton, S. (2014) *The state of the UK's birds 2014*. Sandy, Bedfordshire.

Hayhow, D., Ausden, M., Bradbury, R., Burnell, D., Copeland, A., Crick, H., Eaton, M., Frost, T., Grice, P., Hall, C., Harris, S., Morecroft, M., Noble, D., Pearce-Higgins, J., Watts, O., and Williams, J. (2017) *The state of the UK's birds 2017*. Sandy, Bedfordshire.

Hewson, C. M., Thorup, K., Pearce-Higgins, J. W., and Atkinson, P. W. (2016) Population decline is linked to migration route in the Common Cuckoo. *Nature Communications* 7: 12296.

Honza, M., Øien, I. J., Moksnes, a., and R⊘skaft, E. (1998) Survival of Reed Warbler *Acrocephalus scirpaceus* clutches in relation to nest position. *Bird Study* 45(1): 104–108.

Honza, M., Taborsky, B., Taborsky, M., Teuschl, Y., Vogl, W., Moksnes, A., and Røskaft, E. (2002) Behaviour of female common cuckoos, *Cuculus canorus*, in the vicinity of host nests before and during egg laying: a radiotelemetry study. *Animal Behaviour* 64(6): 861–868.

ICO (2018) *Setzè informe del Programa de Seguiment d'Ocells Comuns a Catalunya (SOCC)*. Barcelona.

Ishizawa, J., and Chiba, S. (1966) Food analysis of four species of cuckoos in Japan. *Miscellaneous Reports of Yamashima Institute of Ornithology* 4: 302–326.

Jacobsen, L. B., Jensen, N. O., Willemoes, M., Hansen, L., Desholm, M., Fox, A. D., Tøttrup, A. P., and Thorup, K. (2017) Annual spatiotemporal migration schedules in three larger insectivorous birds: European nightjar, common swift and common cuckoo. *Animal Biotelemetry* 5(1): 4.

Kålås, J. A., Husby, M., Nilsen, E. B., and Vang, R. (2014) *Bestandsvariasjoner for terrestriske fugler i Norge 1996-2013. Rapport 4-2014*.

Kilner, R. M., Noble, D. G., and Davies, N. B. (1999) Signals of need in parent-offspring communication and their exploitation by the common cuckoo. *Nature* 397: 667–672.

King, R. A., Symondson, W. O. C. and Thomas, R. J. (2015) 'Molecular analysis of faecal samples from birds to identify potential crop pests and useful biocontrol agents in natural areas', *Bulletin of Entomological Research* 105 (3): 261–272

Kleven, O., Moksnes, A., Røskaft, E., and Honza, M. (1999) Host species affects the growth rate of cuckoo (*Cuculus canorus*) chicks. *Behavioral Ecology and Sociobiology* 47(1–2): 41–46.

Kleven, O., Moksnes, A., Røskaft, E., Rudolfsen, G., Stokke, B. G., and Honza, M. (2004) Breeding success of common cuckoos *Cuculus canorus* parasitising four sympatric species of *Acrocephalus* warblers. *Journal of Avian Biology* 35(5): 394–398.

Korzun, L. P., Erard, C., Gasc, J. P., and Dzerzhinsky, F. J. (2003) Biomechanical features of the bill and and jaw apparatus of cuckoos, turacos and the hoatzin in relation to food acquisition and processing. *Ostrich - Journal of African Ornithology* 74(1–2): 48–57.

Koskimies, J. (1947) On movements of the Swift, *Micropus a. apus* L., during the breeding season. *Ornis Fennica* 24: 106–111.

Kuresoo, A., Pehlak, H., and Nellis, R. (2011) Population trends of common birds in Estonia in 1983-2010. *Estonian Journal of Ecology* 60(2): 88.

Laaksonen, T., and Lehikoinen, A. (2013) Population trends of boreal birds: continuing declines in long-distance migrants, agricultural and northern species. *Biological Conservation* 168: 99–107.

Lack, D. (1963) Cuckoo hosts in England. *Bird Study* 10(4): 185–202.

Lack, D. L. (1968). *Ecological adaptations for breeding in birds*. Methuen, London.

Lehikoinen, E. S. A., Sparks, T. H., and Zalakevicius, M. (2004) Arrival and departure dates. *Advances in ecological research* 35: 1–31.

Lindholm, A. K. (1999) Brood parasitism by the cuckoo on patchy reed warbler populations in Britain. *Journal of Animal Ecology* 68(2): 293–309.

Lorimer, J., Sandom, C., Jepson, P., Doughty, C., Barua, M. and Kirby, K.J., (2015). Rewilding: science, practice, and politics. *Annual Review of Environment and Resources* 40: 39-62.

Lormee, H., Boutin, J. M., Pinaud, D., Bidault, H., and Eraud, C. (2016) Turtle Dove *Streptopelia turtur* migration routes and wintering areas revealed using satellite telemetry. *Bird study* 63(3): 425–429.

Lowe, P. R. (1943) Some notes on the anatomical differences obtaining between the Cuculidae and the Musophagidae, with special reference to the specialization of the oesophagus in *Cuculus canorus* Linnaeus. *Ibis* 85: 490–515.

Magrath, R. D. . (1991) Nestling Weight and Juvenile Survival in the Blackbird , *Turdus merula*. *Journal of Animal Ecology* 60(1): 335–351.

Marchetti, K., Nakamura, H., and Gibbs, H. L. (1998) Host-Race Formation in the Common Cuckoo. *Science* 282(5388): 471–472.

Marra, P.P., Hobson, K.A. and Holmes, R.T., (1998). Linking winter and summer events in a migratory bird by using stable-carbon isotopes. *Science* 282(5395): 884-1886.

Marra, P.P., Cohen, E.B., Loss, S.R., Rutter, J.E. and Tonra, C.M., (2015). A call for full annual cycle research in animal ecology. *Biology letters* 11(8): p.20150552.

Martín-Gálvez, D., Soler, M., Soler, J. J., Martín-Vivaldi, M., and Palomino, J. J. (2005) Food acquisition by common cuckoo chicks in rufous bush robin nests and the advantage of eviction behaviour. *Animal Behaviour* 70(6): 1313–1321.

Massimino, D., Woodward, I.D., Hammond, M.J., Harris, S.J., Leech, D.I., Noble, D.G., Walker, R.H., Barimore, C., Dadam, D., Eglington, S.M., Marchant, J.H., Sullivan, M.J.P., Baillie, S.R. and Robinson, R. A. (2017) *BirdTrends 2017: trends in numbers, breeding success and survival for UK breeding birds. Research Report 704.* Thetford.

McAtee, W. L. (1906) The Shedding of the Stomach Lining by Birds. *The Auk* 23(3): 346–346.

McAtee, W. L. (1917) The shedding of the stomach lining by birds, particularly as exemplified by the Anatidæ. *The Auk* 34(4): 415–421.

Medeiros, M. C., and Freed, L. A. (2009). A fledgling-mass threshold greatly affects juvenile survival in the Hawaii akepa (*Loxops coccineus coccineus*). *The Auk* 126(2): 319-325.

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kübler, K., Bissolli, P., Braslavská, O. G., Briede, A., and Chmielewski, F. (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology* 12(10): 1969–1976.

Moksnes, A., and Røskaft, E. (1995) Egg-morphs and host preference in the common cuckoo (*Cuculus canorus*): an analysis of cuckoo and host eggs from European museum collections. *Journal of Zoology* 236(4): 625–648.

Moksnes, A., Fossøy, F., Røskaft, E., and Stokke, B. G. (2013) Reviewing 30 years of studies on the Common Cuckoo: accumulated knowledge and future perspectives. *Chinese Birds* 4(1): 3–14.

Møller, A. P., Rubolini, D., and Lehikoinen, E. (2008) Populations of migratory bird species that did not show a phenological response to climate change are declining. *Proceedings of the National Academy of Sciences* 105(42): 16195–16200.

Møller, A. P., Saino, N., Adamík, P., Ambrosini, R., Antonov, A., Campobello, D., Stokke, B. G., Fossøy, F., Lehikoinen, E., Martin-Vivaldi, M., Moksnes, A., Moskat, C., Røskaft, E., Rubolini, D., Schulze-Hagen, K., Soler, M., and Shykoff, J. a (2011) Rapid change in host use of the common cuckoo *Cuculus canorus* linked to climate change. *Proceedings of the Royal Society B: Biological Sciences* 278: 733–738.

Morrison, K. W., Hipfner, J. M., Gjerdrum, C., and Green, D. J. (2009). Wing length and mass at fledging predict local juvenile survival and age at first return in tufted puffins. *The Condor* 111(3): 433-441.

Moshøj, C. M., Eskildsen, D. P., Jørgensen, M. F., and Vikstrøm, T. (2018) *Overvågning af de almindelige fuglearter i Danmark 1975-2017. Årsrapport for Punkttællingsprogrammet.*

Nakamura, H. (1990) Brood parasitism by the Cuckoo *Cuculus canorus* in Japan and the start of new parasitism on the Azure-winged Magpie *Cyanopica cyana*. *Jap. J. Ornithol.* 39(1): 1–18.

Nakamura, H., and Miyazawa, Y. (1997) Movements, space use and social organisation of radio tracked common cuckoos during the breeding season in Japan. *Japanese Journal of Ornithology* 46: 23–54.

Newson, S. E., Ockendon, N., Joys, A., Noble, D. G., and Baillie, S. R. (2009) Comparison of habitat-specific trends in the abundance of breeding birds in the UK. *Bird Study* 56(2): 233–243.

Newson, S. E., Moran, N. J., Musgrove, A. J., Pearce-Higgins, J. W., Gillings, S., Atkinson, P. W., Miller, R., Grantham, M. J., and Baillie, S. R. (2016) Long-

term changes in the migration phenology of UK breeding birds detected by large-scale citizen science recording schemes. *Ibis* 158: 481–495.

Newton, I. (2004a) Population limitation in migrants. *Ibis* 146(2): 197–226.

Newton, I. (2004b) The recent declines of farmland bird populations in Britain: An appraisal of causal factors and conservation actions. *Ibis* 146(4): 579–600.

Ockendon, N., Hewson, C. M., Johnston, A., and Atkinson, P. W. (2012) Declines in British-breeding populations of Afro-Palaearctic migrant birds are linked to bioclimatic wintering zone in Africa, possibly via constraints on arrival time advancement. *Bird Study* 59(2): 111–125.

Ockendon, N., Johnston, A., and Baillie, S. R. (2014) Rainfall on wintering grounds affects population change in many species of Afro-Palaearctic migrants. *Journal of Ornithology* 155(4): 905–917.

Øien, I., Honza, M., Moksnes, A., and Røskaft, E. (1996) The risk of parasitism in relation to the distance from reed warbler nests to cuckoo perches. *Journal of Animal Ecology* 65(2): 147–153.

Oppel, S., Dobrev, V., Arkumarev, V., Saravia, V., Bounas, A., Kret, E., Velevski, M., Stoychev, S. and Nikolov, S. C. (2015). High juvenile mortality during migration in a declining population of a long-distance migratory raptor. *Ibis* 157(3): 545-557.

Parmesan, C., and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*(6918), 37.

PECBMS (2019) Pan-European Common Bird Monitoring Scheme. Accessed: <https://pecbms.info/. >.

Pompanon, F., Deagle, B. E., Symondson, W. O., Brown, D. S., Jarman, S. N., and Taberlet, P. (2012) Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology* 21(8): 1931–1950.

Pywell RF, Heard MS, Woodcock BA, Hinsley S, Ridding L, Nowakowski M, Bullock JM. (2015) Wildlifefriendly farming increases crop yield: evidence for ecological intensification. *Proc. R. Soc. B* 282: 20151740.

Ramírez, I., Paiva, V. H., Fagundes, I., Menezes, D., Silva, I., Ceia, F. R., Phillips, R. A., Ramos, J. A., and Garthe, S. (2016) Conservation implications of consistent foraging and trophic ecology in a rare petrel species. *Animal Conservation* 19(2): 139–152.

Rey, E. (1892) *Altes und neues aus dem Haushalte des Kuckucks*. Leipzig.

Ringsby, T. H., Sæther, B. E., and Solberg, E. J. (1998). Factors affecting juvenile survival in house sparrow *Passer domesticus*. *Journal of Avian Biology* 29: 241-247.

Robinson, R.A. and Sutherland, W.J. (2002) Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology* 39: 157–176

Robinson, R.A., Wilson, J.D. and Crick, H.Q.P. (2001). The importance of arable habitat for farmland birds in grassland landscapes. *Journal of Applied Ecology*, 38: 1059-1069.

Romanowski, J., and Zmihorski, M. (2008) Selection of foraging habitat by grassland birds: Effect of prey abundance or availability? *Polish Journal of Ecology* 56(2): 365–370.

Rolstad, J., and Rolstad, E. (1995) Seasonal patterns in home range and habitat use of the grey-headed woodpecker *Picus canus* as influenced by the availability of food. *Ornis Fennica* 72(1): 1–13.

Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., and Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature* 421(6918): 57.

Rose, L. N. (1982) Breeding ecology of British pipits and their Cuckoo parasite. *Bird Study* 29(1): 27–40.

Saino, N., Rubolini, D., Lehikoinen, E., Sokolov, L. V, Bonisoli-Alquati, A., Ambrosini, R., Boncoraglio, G., and Møller, A. P. (2009) Climate change effects on migration phenology may mismatch brood parasitic cuckoos and their hosts. *Biology letters* 5(4): 539–541.

Schneider, T., Bischoff, T. and Haug, G.H., 2014. Migrations and dynamics of the intertropical convergence zone. *Nature*, 513(7516): 45.

Seel, D. C. (1977) Trapping Season and Body Size in the Cuckoo. *Bird Study* 24(2): 114–118.

Sharrock, J. T. R. (1976). *The Atlas of Breeding Birds in Britain and Ireland*. Poyser, Calton.

Skjelseth, S., Moksnes, A., Røskaft, E., Gibbs, H. L., Taborsky, M., Taborsky, B., Honza, M., and Kleven, O. (2004) Parentage and host preference in the common cuckoo Cuculus canorus. *Journal of Avian Biology* 35(1): 21–24.

Smith, W. W. (1930) Feeding Habits of the Shining Bronze-Cuckoo. *The Emu* 30(1): 217–218.

Sparks, T. H. (1999) Phenology and the changing pattern of bird migration in Britain. *International Journal of Biometeorology* 42(3): 134–138.

Sparks, T. H., Huber, K., and Dennis, R. L. H. (2006) Complex phenological responses to climate warming trends? Lessons from history. *European Journal of Entomology* 103(2): 379–386.

Sparks, T. H., Huber, K., Bland, R. L., Crick, H. Q. P., Croxton, P. J., Flood, J., Loxton, R. G., Mason, C. F., Newnham, J. A., and Tryjanowski, P. (2007) How consistent are trends in arrival (and departure) dates of migrant birds in the UK? *Journal of Ornithology* 148(4): 503–511.

Stanbury, A., Salter, A., Slader, P., and Tayton, J. (2006) *Breeding Bird Survey of Dartmoor Training Area.* Sandy, Bedfordshire.

Stoate, C., Boatman, N.D., Borralho, R.J., Rio Carvalho, C., de Snoo, G.R. and Eden, P. (2001). Ecological impacts in arable intensification in Europe. *Journal of Environmental Management*. 63: 337-365.

Studds, C. E., and Marra, P. P. (2011) Rainfall-induced changes in food availability modify the spring departure programme of a migratory bird. *Proceedings of the Royal Society of London B: Biological Sciences* : rspb20110332.

Szép, T., Nagy, K., Nagy, Z., and Halmos, G. (2012) Population trends of common breeding and wintering birds in Hungary, decline of long-distance migrant and farmland birds during 1999–2012. *Ornis Hungarica* 20(2): 13–63.

Thorup, K., Tøttrup, A. P., Willemoes, M., Klaassen, R. H. G., Strandberg, R., Vega, M. L., Dasari, H. P., Araújo, M. B., Wikelski, M., and Rahbek, C. (2017) Resource tracking within and across continents in long-distance bird migrants. *Science Advances* 3(1): 1–11.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. and Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature* 418(6898), p.671.

Tinbergen, J. M., and Dietz, M. W. (1994). Parental energy expenditure during brood rearing in the great tit (*Parus major*) in relation to body mass, temperature, food availability and clutch size. *Functional ecology*: 563-572.

Tyller, Z., Kysučan, M., and Grim, T. (2018) Postfledging behavior of the Common Cuckoo (*Cuculus canorus*) attended by the Chaffinch (*Fringilla coelebs*): a comprehensive approach to study the least-known stage of brood parasite–host coevolution. *The Wilson Journal of Ornithology* 130(2): 536–542.

van Wijk, R. E., Schaub, M., Tolkmitt, D., Becker, D., and Hahn, S. (2013) Short-distance migration of Wrynecks *Jynx torquilla* from Central European populations. *Ibis* 155(4): 886–890.

Vega, M. L., Willemoes, M., Thomson, R. L., Tolvanen, J., Rutila, J., Samaš, P., Strandberg, R., Grim, T., Fossøy, F., Stokke, B. G., and Thorup, K. (2016) First-Time Migration in Juvenile Common Cuckoos Documented by Satellite Tracking. *PLOS ONE* 11(12): e0168940.

Vickery, J. A., Tallowin, J. R., Feber, R. E., Asteraki, E. J., Atkinson, P. W., Fuller, R. J., and Brown, V. K. (2001) The management of lowland neutral grasslands in Britain: effects of agricultural practices on birds and their food resources. *Journal of Applied Ecology* 38(3): 647–664.

Vickery, J. a., Ewing, S. R., Smith, K. W., Pain, D. J., Bairlein, F., Škorpilová, J., and Gregory, R. D. (2014) The decline of Afro-Palaearctic migrants and an assessment of potential causes. *Ibis* 156(1): 1–22.

Vogl, W., Taborsky, M., Taborsky, B., Teuschl, Y., and Honza, M. (2002) Cuckoo females preferentially use specific habitats when searching for host nests. *Animal Behaviour* 64: 843–850.

Vogl, W., Taborsky, B., Teuschl, Y., Taborsky, M., and Honza, M. (2004) Habitat and space use of European cuckoo females during the egg laying period. *Behaviour* 141(7): 881–898.

Waring, P., and Townsend, M. (2017) *Field guide to the moths of Great Britain and Ireland*. Bloomsbury Publishing.

Welch, A.J., Fleischer, R.C., James, H.F., Wiley, A.E., Ostrom, P.H., Adams, J., Duvall, F., Holmes, N., Hu, D., Penniman, J. and Swindle, K.A., (2012). Population divergence and gene flow in an endangered and highly mobile seabird. *Heredity* 109(1): 19.

Whiteman, C.D., (2000). *Mountain meteorology: fundamentals and applications*. Oxford University Press.

Wiley, A.E., Rossman, S., Ostrom, P.H., France, C.A., Penniman, J., Bailey, C., Duvall, F., Zipkin, E.F. and James, H.F., (2019). From ecologically equivalent individuals to contrasting colonies: quantifying isotopic niche and individual foraging specialization in an endangered oceanic seabird. *Marine Biology* 166(3): 39.

Willemoes, M., Strandberg, R., Klaassen, R. H. G., Tøttrup, A. P., Vardanis, Y., Howey, P. W., Thorup, K., Wikelski, M., and Alerstam, T. (2014) Narrow-front loop migration in a population of the common cuckoo *Cuculus canorus*, as revealed by satellite telemetry. *PloS one* 9(1): e83515.

Williams, G., Henderson, A., Goldsmith, L. and Spreadborough, A., (1983). The effects on birds of land drainage improvements in the North Kent Marshes. *Wildfowl*, 34(34): 33-47.

Williams, H. M., Willemoes, M., Klaassen, R. H. G., Strandberg, R., and Thorup, K. (2016) Common Cuckoo home ranges are larger in the breeding season than in the non-breeding season and in regions of sparse forest cover. *Journal of Ornithology* 157(2): 461–469.

Wyllie, I. (1981) *The Cuckoo*. London: Batsford.

Zühlke, E.-M. (2009) *The Occupancy of Conifer Plantations and Felled Conifer Plantations by Bird Species in the Process of Blanket Bog Restoration in the Flow Country, North Scotland*. Tampere Polytechnic.

2. General materials and methods

In order to identify and test hypotheses on breeding season habitat, host and food resources of the common cuckoo *Cuculus canorus*, field data collection was carried out between 2015 and 2017 within an upland study area on Dartmoor in Devon, UK which holds a population of cuckoos parasitizing nests of predominantly meadow pipits *Anthus pratensis* in semi-natural grassland. The field study took place predominantly at the site of an existing nest monitoring project (established 2008) targeting nests of all breeding bird species within 2 m height from the ground (Dartmoor Upland Bird Nest Group, 2017). As meadow pipits were the most frequent cuckoo host as observed by the nest monitoring project, host nest fieldwork for the current study was focused exclusively on this species. Diet of cuckoo adults and cuckoo and host nestlings were studied by collecting faecal samples from free-flying or mist-netted adult cuckoos and from nestlings at the nest, and analysing samples after the breeding season using a laboratory-based molecular sequencing approach.

Field methods

Study area and study sites

All field study sites were located within Dartmoor National Park, Devon, UK (Fig. 1). The area comprises largely semi-natural grass moor (often with gorse *Ulex*) and heath, and a significant extent of improved grassland. Woodland (ancient broadleaf) and forested areas (conifer plantation) have a scattered distribution within the lower altitudes (largely below 300 m) with mostly individual or small groups of rowan *Sorbus aucuparia* and hawthorn *Crataegus monogyna* trees above 300 m. The area represents both a remaining stronghold for cuckoo in Devon (Beavan & Lock 2016) and the southwest region of England (Balmer *et al.* 2013), and an area of mixed upland land-use in which to examine correlates of cuckoo and host resources with land use. Holne Moor (50° 31’ 20” N, 03° 51’ 43” W, altitude 300-400 m) was the primary study area for fieldwork on meadow pipits including all nests parasitised by cuckoo included in the study. An existing

monitoring programme of the nests of all ground and shrub nesting bird species (including meadow pipit and cuckoo) was carried out at this site between mid-March and the end of July every year, between 2008 and 2017 inclusive. The site covered roughly 200 ha of *Festuca ovina-Agrostis capillaris-A. curtisii* grassland surrounding a well-wooded artificial reservoir with variable gorse *Ulex* growth, in some areas rotationally burned in rectangular blocks. The moor was scattered with trees, mainly hawthorn and rowan, and bordered to the east by improved pastoral grassland agriculture and to the north by native ancient oak *Quercus* and birch *Betula* woodland. It received mixed

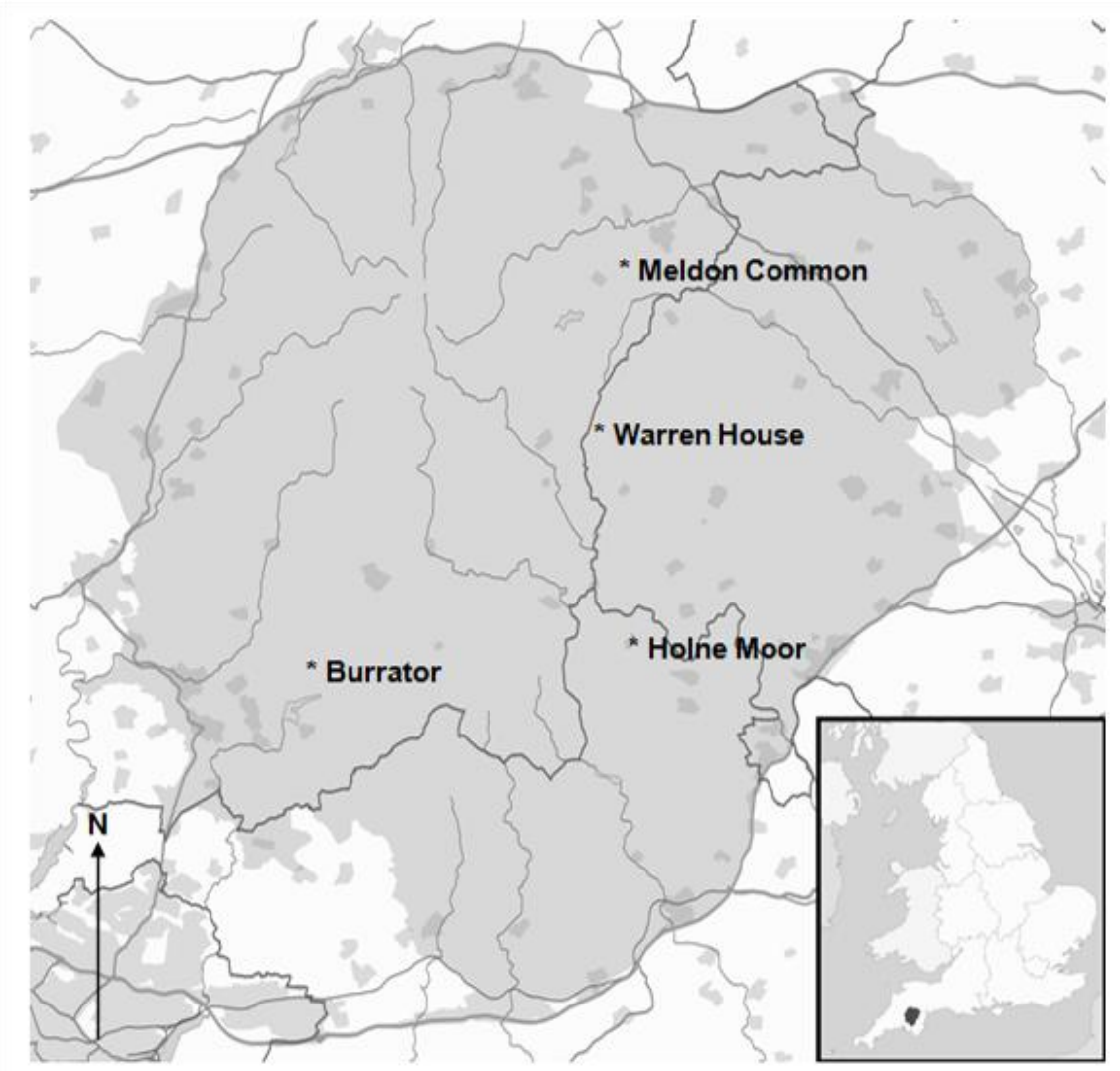


Figure 1. Study sites within Dartmoor National Park (light grey shaded), and location of Dartmoor in south-west UK (inset, black shaded).

grazing from sheep, cattle and ponies that varied in intensity during the bird breeding season as it was accessible to several members of a commoners association. Meldon Common (50° 39' 40" N, 03° 50' 52" W, altitude 300-400 m) was selected as an area of moorland edge. It had a similar grassland assemblage to Holne Moor, with gorse burnt in rotational blocks and scattered rowan trees. It was less than 100 ha in size and surrounded in all directions by improved pastoral grassland agriculture. During the breeding season it received grazing of variably intensity from ponies overseen by a commoners association. Warren House (50° 36' 43" N, 03° 52' 13" W) and Burrator (50° 31' 24" N, 04° 0' 55" W) were selected as areas with high encounter rate and density of common cuckoo (pers. obs., Beaven and Lock 2016) at which to collect faecal samples of adult cuckoos. Warren House covered an area of roughly 100 ha of heath and mire near a large plantation of Sitka spruce *Picea sitchensis*. The heath was grazed by sheep during the bird breeding season. Burrator covered roughly 100 ha of a valley with well-grazed *Festuca ovina-Agrostis capillaris-A. curtisii* grassland and *Molinia caerulea* mire grassland, near a Sitka spruce plantation. The dominant tree species was hawthorn, and the site was grazed by sheep and cattle in the bird breeding season.

Nest finding, monitoring and brood handling procedures

Nest monitoring for the present study took place at Holne Moor in 2015 to 2017, at Meldon Common in 2015 and 2016, and at Warren House and Burrator in 2015 only. Meadow pipit nests were found by non-random searching of each site from 06:00 to 17:00 at least two days per week from the start of April to the end of July, in routes that maximised coverage of whole sites on each visit day. To locate nests, bird behaviour was observed for evidence of nest building or returning to nest to incubate or feed young or the incubating female. Whilst walking site routes, vegetation was also tapped constantly to also detect nests in proximity by flushing of incubating females. In order to relocate nests on subsequent visits, dwarf shrub vegetation within 1 m of the nest cup was marked at the top with a 2 cm 'flag' of red insulating tape (chosen because red is less conspicuous to nest predators) and their location was recorded as 10-digit British National Grid co-ordinates using a handheld GPS (accuracy = ±3 m) (Garmin, Lenexa, USA). From detection of a nest until the nest attempt ended

due to fledging or failure, nest contents (numbers of eggs and young of pipit or cuckoo) were recorded every 3 to 4 days both for the present study and the British Trust for Ornithology (BTO) Nest Record Scheme. To record chick feather growth stage that could be more readily compared between host and cuckoo nestlings, feather stage was recorded as naked (NA), flight feathers in pin (IP), flight feathers up to one third grown (FS), or flight feathers over one third grown (FM+). For broods varying in feather stage, the modal (majority) category was recorded. Chick age in days was largely estimated from appearance but was estimated from hatching date when this was known.

In order to identify diet of cuckoo and meadow pipit nestlings, faecal sacs were collected from nestlings of both species in meadow pipit nests at field sites, for analysis by molecular sequencing in the laboratory. To collect faecal sacs from nestlings, some of the above monitoring visits were made under Natural England license (2015-9819-SCI-SCI-1 and 2017-28736-SCI-SCI), a maximum of two times between the nestling ages of 4 to 10 days (meadow pipit) or 3 to 16 days (cuckoo). Faecal samples of the whole brood of meadow pipits or the single cuckoo nestling in the nest were captured into a single 5 ml screw-top vial per sampling visit to the nest. In order to reduce possible cross-contamination between faecal samples, the vial was handled with a clean latex glove, and birds were handled to position the cloaca over the opening of the vial and directly capture a faecal sac as it was released. Sacs dropped onto other surfaces were collected into the vial by moving the vial underneath and rolling the sac into it by angling the surface or using a sterile Pasteur pipette. Wearing a clean pair of latex gloves, a sterile Pasteur pipette was used to transfer 80-100% ethanol from a stock vial to the sample vial until all material was covered. The Pasteur pipette was used to pierce each faecal sac to allow ethanol to penetrate, and the vial lid was fitted. The vial was marked with date, nest identity, species of nestling, and fraction of brood sampled using an alcohol-proof permanent marker, double-bagged in plastic and stored within 24 h at 5° C. However, following the present study it is recommended that samples are stored as cold as possible, e.g. -80° C, to minimize DNA degradation, and that 100% ethanol is used to preserve samples if refrigeration will be delayed. Used gloves and pipettes were sealed in plastic bags to reduce contamination risk.

In order to test hypotheses on nestling body condition of meadow pipits, for each handled nestling the total tarsus length was measured to 0.1 mm with callipers (Wiha, Schonach im Schwarzwald, Germany) and body mass was measured to 0.25 g with a 30 g spring balance (accuracy ±0.3%) (Pesola, Feusisberg, Switzerland). In order to identify individual nestlings, nestlings at Holne Moor were individually marked with a numbered metal ring as part of the BTO Ringing Scheme. At Meldon Common, individuals were temporarily marked by colouring each nestling (maximum brood size = 5) in one of the following ways with ink: i) left tibia, ii) right tibia, iii) both tibias, iv) neither tibia, or v) striped on both tibias. This technique was also used to identify individuals at both sites when nestlings were not sufficiently developed to be fitted with rings. The visit to fit rings to nestlings was sometimes conducted in addition to the two Natural England licensed visits. These were carried out under BTO Ringing Scheme license and faecal sacs released during this handling were collected for the present study under the ringing license, following the sampling protocol above.

Parental provisioning observation

In order to quantify adult meadow pipit provisioning of nests containing a meadow pipit brood or a cuckoo nestling, observation sessions were carried out a maximum of once per day on each meadow pipit nest containing a single cuckoo nestling or a brood of meadow pipit nestlings. Because fledgling cuckoos stayed in one location for long periods after leaving the nest, observations were also carried out on fledgling cuckoos identically to nests. In order to capture potential trade-offs that may cause nest provisioning visit rate to vary, observation sessions were designed to also capture prey load size delivered, foraging distance from the nest that adults collected food, and the locations where adults collected food (which could be subsequently surveyed to measure habitat use). Observation sessions were carried out between May and July, in the years 2016 and 2017 at Holne Moor, and in 2016 at Meldon Common. During the period that cuckoo nestlings or fledglings were present at Holne Moor (late May to end of July), all cuckoo nestlings and fledglings received observation sessions, and nests containing meadow pipit broods at Holne Moor were selected for observation on the criteria of how similar in i)

geographic location and ii) nestling feather growth stage they were to a nest containing a cuckoo nestling, or a fledged cuckoo. At Meldon Common, no nests with cuckoo nestlings were located, and all nests with pipit nestlings received observation sessions. In order to reduce observer variation in data, I carried out all observation sessions in the study. Vantage point distance from the nest varied from 33 to 261 m. In order to allow simultaneous measurement of provisioning visit rate, prey load size, foraging distance and foraging habitat use, vantage points were selected to enable a view of maximum radius surrounding the nest in all directions, while also allowing closest possible examination of bill contents of returning adult pipits by telescope (as in Douglas *et al.* 2008). Vantage point was also selected on availability of cover or skyline against which to be concealed from birds using a camouflage hide. Vantage point height above ground varied from 0.5 to 1.5 m. In order to minimise observer disturbance effects on provisioning behaviour, observation sessions took place more than one hour after the nest had been visited to check contents or collect samples, and more than one hour after the nest was first found.

Observations of maximum duration 60 mins were carried out between 06.00 and 19.00, and were considered to be in progress once no alarm calls were being made by adult pipits. In order to reduce disturbance effects of the observation session, the hide with telescope inside was set up at least 10 min before observation and, through delaying starting observations and through the distance of the vantage point, alarm calling by birds was typically not evident. Once a session had started, pipits alarm calling and approaching to the hide triggered observation to be aborted, but alarm calling without approach was simply noted, and was usually associated with passing predatory species or other non-observer disturbance. In order to readily detect birds returning to the nest from foraging, the default field of view throughout observation sessions was a 20 to 40 m radius around the nest site, usually by unaided vision or binoculars. A telescope was trained on the nest location. On arrival of a provisioning bird to the nest site (<4 m from the nest cup), the direction of origin was noted and a telescope view was adopted on the bird carrying food. In order to quantify prey load size, prey load length was estimated relative to the pipit's bill length (0.25x, 0.50x, 0.75x, 1.00x, then increments of 0.50x thereafter).

Time of arrival was noted, binoculars were used to watch the bird into the nest, and in order to successfully track the exiting bird from the nest to its next foraging location, binocular view was fixed on this position until the bird left the nest. On exit, the bird was kept in view to landing on the ground or (very rarely) starting to collect food from raised foliage. Birds were normally lost from view on the ground but if not were always seen to forage shortly after. Birds that landed in raised foliage generally did not collect food until dropping to ground or flying further to a ground patch (normally after just a few seconds unless resting or preening). Ground patches where birds landed and did not fly up within a few seconds were therefore assumed to be foraging locations. Time of nest exit was recorded, and a description of the location ('foraging plot') to which the bird had flown was recorded; before returning to broadest field of view to watch for the next visit. Times that the nest was exited were used as the point between which visit rates could be calculated, as time spent out of view entering the nest on arrival varied, and birds sometimes dropped at distance from the nest preventing identification of when the nest was entered. However if a bird entered the nest area with food but was not seen to leave after 2 min (often relating to brooding young) then for the purpose of calculating feeding rate, birds were assumed in these cases to have delivered food at time of entry, not exit, and in this instance the time of arrival was used.

Over the course of observation, foraging plots of origin and of destination were recorded for as many feeding visits as possible: plots visited following a visit with food to the nest, were more easily identified than plots from which birds had arrived, as observer focus was placed on detecting birds visiting the nest over birds re-emerging from foraging plots. In order to measure distance and allow re-visiting of foraging plots for habitat surveying, newly identified foraging plots were visited immediately after an observation session ended to record their location with a handheld GPS (accuracy ±3 m) (Garmin, Lenexa, USA). Identified foraging locations were considered to be distinct foraging plots if found during GPS recording to be more than 4 m apart. The 10-digit British National Grid references of the nest and identified foraging plot were later used to calculate linear foraging distance (in metres) of each foraging plot from the nest, using the Pythagorean equation:

Distance = $\sqrt{(x^2 + y^2)}$

where x = change in 5-digit x co-ordinate, and y = change in 5-digit y co-ordinate, both of which are the equivalent length in metres. Calculating straight-line distance was considered representative of actual feeding behaviour due to the linear flights taken by observed birds. Foraging plots were largely identifiable up to 200m from the nest. Birds sometimes flew beyond the viewshed and landed in an unidentifiable area. In these cases, distance was logged as >200m and habitat data for these plots was not collected. Observations terminated after 60 min or when a fifth identifiable foraging plot was used by the foraging adults; whichever was soonest. Due to proximity of foraging plots to active nests, visits to take GPS locations were brief and habitat recording on foraging plots took place after peak nesting season was over.

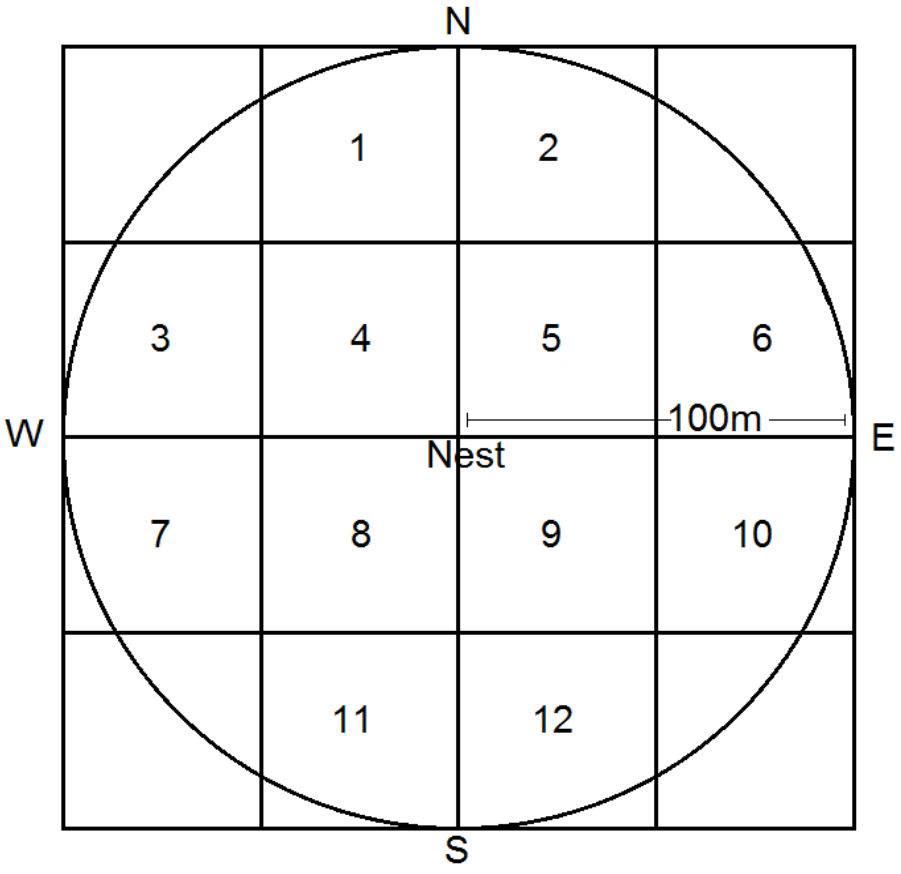


Figure 2. Alignment of habitat survey squares 1-12 overlying a 100m radius around the nest cup.

Table 1. Names, descriptions and shorthand codes of habitat variables surveyed around nests and on 10 x 10 m patches used for foraging by meadow pipits.

Variable	CODE
Low gorse *Ulex spp.* (<75 cm)	LG
Medium gorse (75-150 cm)	MG
Mixed gorse and tufted grass (<75cm)	GG
Low homogeneous grasses (<10 cm)	GR
Grazed pastoral grass	PG
Long homogeneous-height grasses, i.e. hay meadow (>10 cm)	HO
Tufted long grasses >10 cm	TU
Dead/burnt gorse stalks and short grass <10 cm	DG
Live bracken	BR
Heather	HE
Bilberry	BI
Mosses	MO
Bare rock	RK
Bare ground/earth	BGRD
Juncus	JU
Bog	BO
Open water (still or flowing)	OW
Shrubs ≥ 1.5 m high	SHRUB*
Trees ≥1.5 m high	TREE*
Presence of shrubs ≥ 1.5 m high	PRESSHRUB
Presence of trees ≥1.5 m high	PRESTREES
Presence of trees ≥3 m high	PRESTREES3

Habitat recording

In order to quantify fine-scale habitat of meadow pipit nesting and foraging habitat, vegetation and substrate cover was surveyed in 12 50 x 50 m squares within the 100m radius around the nest (Fig. 2). 100 m was previously reported in the literature to be a radius within which most meadow pipit foraging took place (Douglas *et al.* 2008). For each 50 m square, estimated percentage cover of each vegetation or substrate type was used to give ordinal scores of 0 (absent), r (0-5% single or rare), + (0-5% few), 1 (0-5% numerous), 2 (5-25 %), 3 (25-50 %), 4 (50-75 %) and 5 (75-100 %). This was recorded for the following vegetation types: low gorse *Ulex* (up to 75 cm high), medium gorse (75 to 150 cm high), mixed grasses and gorse (up to 75cm), low homogeneous grasses (up to 10cm sward), long homogeneous grass (over 10cm), tufted grasses (over 10cm), dead gorse stalks with low grass under 10cm, live bracken, dead bracken, heather, mosses, conifers, bare rock/road, bare ground, bilberry, brambles, rushes and open water (streams, aqueducts and pools). The height, width and length of each gorse shrub over 150 cm high was measured, as was the height of each tree over 150 cm high.

Adult cuckoo faecal sampling

In order to identify diet invertebrates of adult cuckoos in the study area, faecal samples were collected from adult cuckoos at field sites for analysis via molecular sequencing in the laboratory. Many faecal sampling studies of adult birds involve capturing individuals in mist-nets (e.g. King *et al.* 2015). Cuckoos are difficult to capture in mist-nets due to their soft plumage, extremely short tarsi and long wings (Noakes 2013). In order to collect an extensive sample of faeces from adult cuckoo for diet analysis, I used a non-capture (non-invasive) approach where fresh faeces were collected at sites that were suitable for tracking birds on foot and recovering dropped faeces. This was supplemented with a mist-netting effort where faeces were collected from adult birds during handling.

i) Non-invasive collection

Faecal sampling from non-captured birds in the field was carried out at Warren House and Burrator between April and June 2017. It was also attempted between April and June in 2015 at Holne Moor, Warren House, Burrator and Meldon Common, and in 2016 at Holne Moor and Meldon Common before the methodology and best site characteristics were fully established. Birds were searched for at sites selected for a combination of high encounter rate of adult cuckoos; terrain that allowed high visibility and accessibility for approaching birds on foot; and suitable substrate for locating and retrieving dropped faecal material of suitable mass for molecular analysis (>100mg). Sampling from non-captured birds meant marking and recognition of individuals was not possible. Samples collected non-invasively therefore originated from an uncertain number of individual birds at two sites.

Searching for cuckoos and non-invasive faecal sampling were carried out from late April when birds were first detected on site until early June when calling and adult activity became less evident. Each located bird was kept in view and followed on foot if necessary, to the point of approaching to retrieve a sample or the bird vacating the site or being lost to view. Visually located perched birds were prioritised over subsequently detected birds, therefore sampling was more efficient when carried out in pairs or groups. Individuals were watched continuously with binoculars when perched, with exact location and orientation on perch being noted, until faecal material was dropped. Volume, composition and trajectory of expulsion were visually assessed, as was suitability of perch position and underlying substrate to finding material; to determine whether to approach and attempt to collect faecal samples, or continue to monitor the bird. Defecations took place approximately every 15 min under typical observed feeding conditions. Faeces were collected a maximum of 30 min after release, dependent partly on likelihood of current weather conditions to cause desiccation of material (humidity, wind, temperature). Dropped faecal material was located by approaching the perch with continuous visual focus on the bird's position at time of defecation. An area of radius 2 m around the point directly below the bird's position was searched methodically for fresh faecal material, while considering wind direction and the bird's orientation. Due to the likelihood

of found material originating from other species (or other individual cuckoos), all fresh faeces located were collected separately from each other into 5 ml screw-top vials, using a clean sterile Pasteur pipette to handle each sample and wearing clean sterile latex gloves when collecting each sample. A sterile Pasteur pipette was used to add 80-100% ethanol to each vial sufficiently to cover faecal material. Vials were marked with date, time, and site using an alcohol-proof permanent marker, double-bagged in plastic and refrigerated at 5° C within 24 h. However, following the present study it is recommended that samples are stored as cold as possible, e.g. -80° C, to minimize DNA degradation, and that 100% ethanol is used to preserve samples if refrigeration will be delayed. Faeces that were small, disintegrated or liquid in form were difficult to retrieve and unsuitable for laboratory processing. Identification of cuckoo faeces among collected samples was achievable by post-hoc molecular analysis in the laboratory. For a minority of samples, identification of origin as cuckoo was possible in the field from presence of caterpillar hairs on the surface or from exact matching of ground position to cuckoo perch position by staking the ground and re-checking from the original vantage point.

ii) Sampling from mist-netted birds

Trapping was carried out at Holne Moor between early May and early June in 2016 and 2017, under BTO Ringing Scheme license with endorsements for tape luring and unconventional trapping methods. Following Noakes (2013) three 18 m mist-nets (30 x 30 mm mesh) (Ecotone, Sopot, Poland) were set in a triangle around a decoy female bird. The decoy comprised a plastic thrush (Turdidae) decoy, repainted in female cuckoo plumage, with attached wings and tail from a taxidermy cuckoo specimen. Adjacent to the mist-nets, a clap net was positioned at the top of a 2 m pole. A sound lure of mixed male and female calls was played below the decoy in two minute bursts with ten minute breaks between. Traps were active from before dawn (c. 04:00 BST) to late morning, and all traps were monitored constantly from around 120 m away when active. Captured birds were extracted from nets immediately and placed in a small-animal carry case covered with blackout material for ten minutes. In order to collect faeces, the holding case was floored with a sheet of laminated cardboard, replaced for each individual bird. All faecal material from an

individual was collected into a 5 ml screw-top vial using a sterile Pasteur pipette and wearing clean latex gloves to sample each bird. A sterile Pasteur pipette was used to add 80-100% ethanol to the vial to cover all faecal material. Each vial was marked with date, time, site and ring number using an alcohol-proof permanent marker, double-bagged in plastic and refrigerated at 5° C within 24 h. However, following the present study it is recommended that samples are stored as cold as possible, e.g. -80° C, to minimize DNA degradation, and that 100% ethanol is used to preserve samples if refrigeration will be delayed. In order to recognise individuals, birds were fitted with a numbered BTO metal ring. Age and sex were identified from plumage characteristics. Measurements were taken of maximum wing chord and weight before release.

Laboratory methods

Laboratory overview

In order to confirm whether samples collected via the non-capture protocol originated from cuckoos, and identify invertebrates present in faecal samples collected in the field from adult cuckoos and cuckoo or host nestlings, samples were processed in the laboratory. DNA was extracted from faecal samples, specific DNA sequence regions were amplified, and sequences were read and matched to sequences of known bird and invertebrate species, respectively. The range of laboratory tasks carried out largely related to identifying prey taxa from remains in faeces of the cuckoo adults and young, and comparison to prey composition of host (meadow pipit) young.

The broad phases of work were: i) extraction of DNA from faecal samples collected in the field; ii) testing ability of extant primer sequences to amplify the DNA sequences required for bird or prey taxon identification; iii) DNA amplification; iv) separation and extraction of prey sequences from the assemblage in each faecal sample; v) identification of prey via sequencing. All samples collected from birds without handling required confirmation of bird species via tasks i) to iii). I identified prey composition of meadow pipit nestlings via a ‘clone and [Sanger] sequence’ approach comprising tasks i) to v). I

identified prey composition of cuckoo nestlings, fledglings and adults, plus some comparison meadow pipit nestlings, via a 'next-generation sequencing' approach comprising tasks i) to v), wherein task iv) is carried out at a high rate within the sequencing apparatus. Testing and validating materials and methods prior to these processes required sourcing of faecal samples of known origin.

Collection of reference avian faeces

Faecal samples collected from non-trapped cuckoos required confirmation of bird species of origin before they could proceed into sequencing for dietary analysis. To confirm that molecular analytical methods (including primer pairs) were suitable for identifying bird species originating each sample, I collected faecal samples of known origin around the University of Exeter campus (50° 44' 8" N, 03° 32' 6" W), from bird taxa which utilise similar perches to cuckoo on the Dartmoor study sites: These were common wood pigeon *Columba palumbus*, carrion crow *Corvus corone* and Eurasian magpie *Pica pica*. Faeces observed to be deposited by these species on hard substrate or short grass were collected into 5 ml screw-top vials using a sterile Pasteur pipette, covered with 90% ethanol and stored immediately at 5° C.

Collection of reference invertebrate tissue

In order to assess how quantity of DNA extracted from different invertebrate taxa proceeded through extraction and sequencing, DNA was extracted from tissue of six invertebrate species of known genomic DNA sequence. The minimum number of individuals was used that would provide sufficient tissue mass for successful DNA extraction (<25 mg). Individuals were humanely destroyed by freezing at -20° C.

Primer selection for DNA amplification

In order to confirm a method for identifying faeces of cuckoo origin from non-target faeces, the ability of DNA primer pairs to amplify the DNA of a range of bird species was compared. Primers targeting the mitochondrial DNA (mtDNA) gene cytochrome-b oxidase I (COI) were tested, both for identifying bird species of origin for samples collected under cuckoo perches, and for identifying

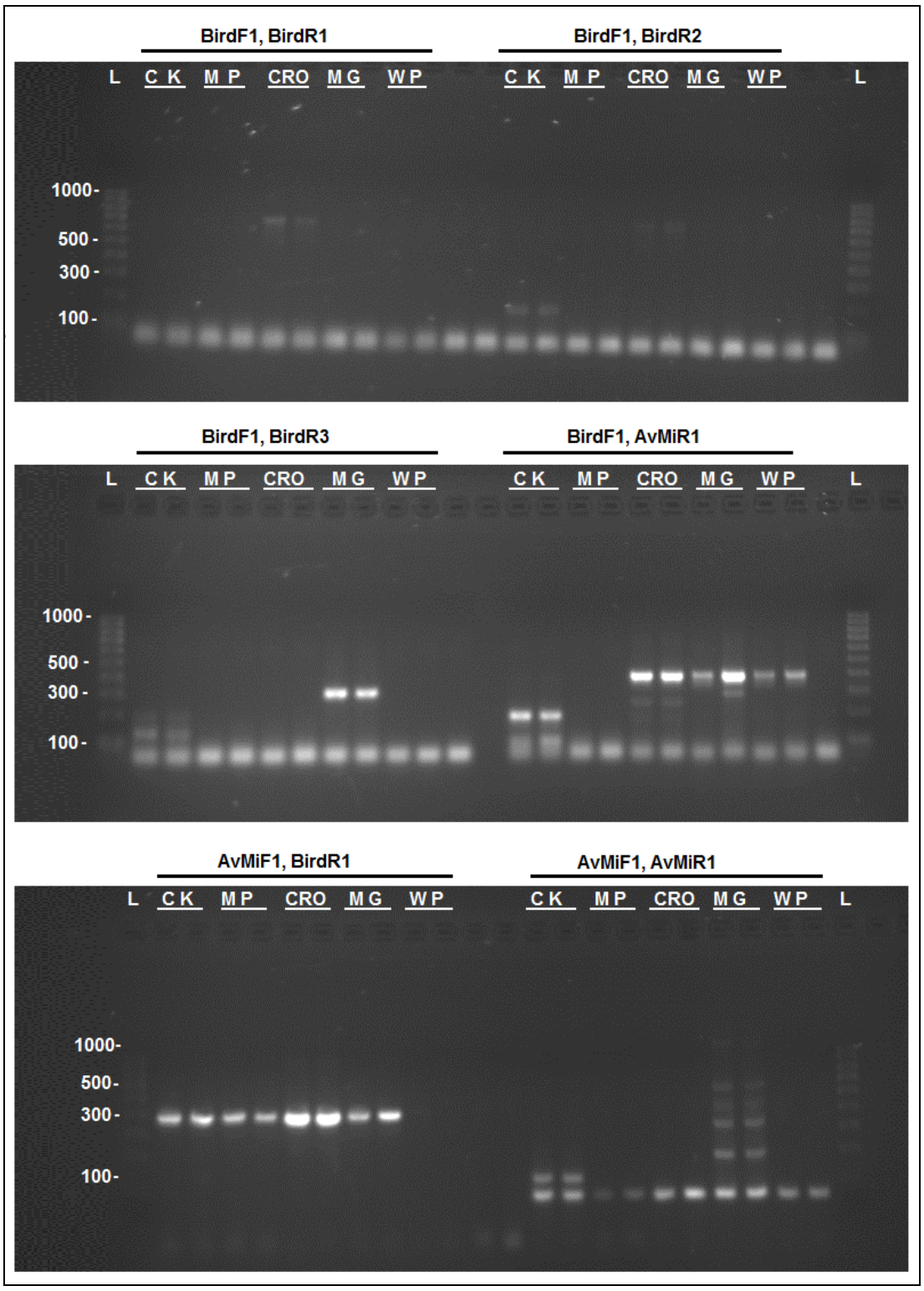


Figure 3. Gel image showing amplification of avian COI DNA from faeces of cuckoo CK, meadow pipit MP, carrion crow CRO, magpie MG and woodpigeon WP, by the primer pairs BirdF1/BirdR1, BirdF1/BirdR2, BirdF1/BirdR3, BirdF1/AvMiR1, AvMiF1/BirdR1, and AvMiF1/AvMiR1. L = allele ladder, numbers are molecule length in base pairs bp.

invertebrate prey from remains in avian faeces. The COI sequence is highly conserved between species but is sufficiently variable in sequence (Brown 1985). As a result, it has become the focus of efforts to document and identify 'DNA barcodes' (Hebert *et al.* 2003) from animal species' genomes. First, primer pairs were assessed in their success in amplifying avian DNA extracted from faecal samples from cuckoo, meadow pipit, carrion crow, (common) magpie and (common) wood pigeon. The primer pairs BirdF1 with BirdR1, and with BirdR2 (Hebert *et al.* 2004), target a relatively long (>700 bp) region of the avian COI gene, and did not significantly amplify DNA of the relevant length from faeces sampled (Fig. 3). BirdF1 with BirdR3 (Hebert *et al.* 2004) targets a shorter ~300 bp region but did not amplify DNA of relevant length from most species' samples (Fig. 3). AvMiF1 and AvMiR1 are, respectively, forward and reverse primers, designed for use in conjunction with reverse or forward primers of the above longer-region designs, to amplify shorter regions (~300-400 bp) (Kerr *et al.* 2009) more likely to remain intact in degraded DNA as found in faeces (Lijtmaer *et al.* 2012). AvMiF1 with BirdR1 amplified DNA of relevant length in all samples except wood pigeon, while BirdF1 with AvMiR1 did not amplify DNA from cuckoo and meadow pipit samples as it did from other tested species (Fig. 3). AvMiF1 and BirdR1 were therefore selected for amplifying and sequencing tasks including confirming avian species as common cuckoo for all faecal samples collected with and without capture (see field methodology). Visual alignment of cuckoo and meadow pipit COI sequences accessed from gene databases, and comparison with published primer sequences, also indicated suitability of Bird F1, Bird R1, AvMiF1 and AvMiR1 for amplifying parts of the COI sequence in Cuckoo and Meadow pipit (Appendix 1).

A nested two-step approach was used for amplification of invertebrate COI DNA from bird faecal samples: The generic invertebrate COI primers LCO1490 and HCO1777 (Folmer *et al.* 1994, Brown et al 2012), targeting a 210 bp region of the COI sequence, amplified DNA of all reference invertebrates tested, but also amplified DNA of Cuckoo and Meadow pipit, causing presence of consumer DNA in downstream analyses which were usually only detected at final sequencing. Invertebrate primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al.* 2011) amplified DNA of all test invertebrates including i) arthropods and ii)

annelid worms, without amplifying reference DNA from cuckoo or meadow pipit. However this primer pair subsequently demonstrated low success in amplifying DNA extracted from faecal samples, likely due to the low concentration of DNA obtained from faeces (range mainly 1 to 10 ng/µl). Alignment of reference invertebrate sequences from gene databases, and comparison with published sequences for both primer pairs, showed the primer binding sites for ZBJ-ArtF1c and ZBJ-ArtR2c lie within the amplification region between the LCO1490 and HCO1777 binding sites (Fig. 4). This indicated that amplified fragments in completed PCR (polymerase chain reaction) using LCO1490 and HCO1777 can be subsequently amplified in PCR reaction with ZBJ-ArtF1c and ZBJ-ArtR2c. The first PCR reaction amplified COI DNA from both consumer and prey, with the primer pair in the nested second PCR reaction subsequently amplifying only invertebrate (prey) COI sequences.

Protocol for preparing pipit nestling faeces for Sanger sequencing

DNA extraction

DNA was extracted from tissue of reference invertebrates using the DNEasy Blood and Tissue kit (Qiagen. Hilden, Germany) following manufacturers' instructions. High-chitin tissue samples entered into extraction exceeded the recommended 15 mg maximum tissue mass, to account for mass of poorly-digestible chitin. The QIAamp DNA Stool Mini kit (Qiagen, Hilden, Germany) was used to extract DNA from reference avian faeces and meadow pipit nestling faeces for clone and sequencing analysis. DNA extraction was carried out with a maximum batch size of 12 samples including 1-2 negative controls (180 µl sterilised molecular biology grade water). for detection of cross-contamination risk. The kit was used following manufacturers' protocol for extraction of non-human DNA, including all recommended steps and with the following alterations: Optimal treatment of samples ahead of extraction was freeze-drying followed by storage at -20°C. Mass of samples entered into extraction varied from 47 – 220 mg. Sample mass exceeded the recommended 220 mg where possible to allow for significant presence of sediment in faeces, noted during homogenisation of the first samples processed. Sample mass was commonly below recommended minimum 180 mg. Initial homogenisation in

buffer ASL was carried out using the Tissuelyser II (Qiagen, Hilden, Germany) with one 5 mm diameter steel bead (Qiagen, Hilden, Germany) per sample tube. When transferring supernatant from the second centrifugation following reaction with InhibitEX tablets, the maximum volume obtainable without pellet material was transferred, rather than recommended 200 µl. A 1:1 ratio of Buffer AL, and later ethanol, was added to this volume during lysis. 15 µl of proteinase K was used for supernatant volumes up to 200 µl, 20 µl for 250-400 µl and 25 µl for 450-600 µl. DNA was eluted from the QIAamp spin column in 50 µl Buffer AE at 95°C, with incubation at room temperature for 5 min before centrifugation/elution. Concentrations of eluted DNA were quantified within a Qubit high-sensitivity (HS) dsDNA assay (Thermo Fisher, USA), which compares fluorescence of samples bound in solution to DNA-specific dye. The range of original DNA solution concentrations detectable by this assay is 10 pg/µl to 100 ng/µl. DNA concentration extracted using the kit was predominantly in the order 1 to 10 ng/µl.

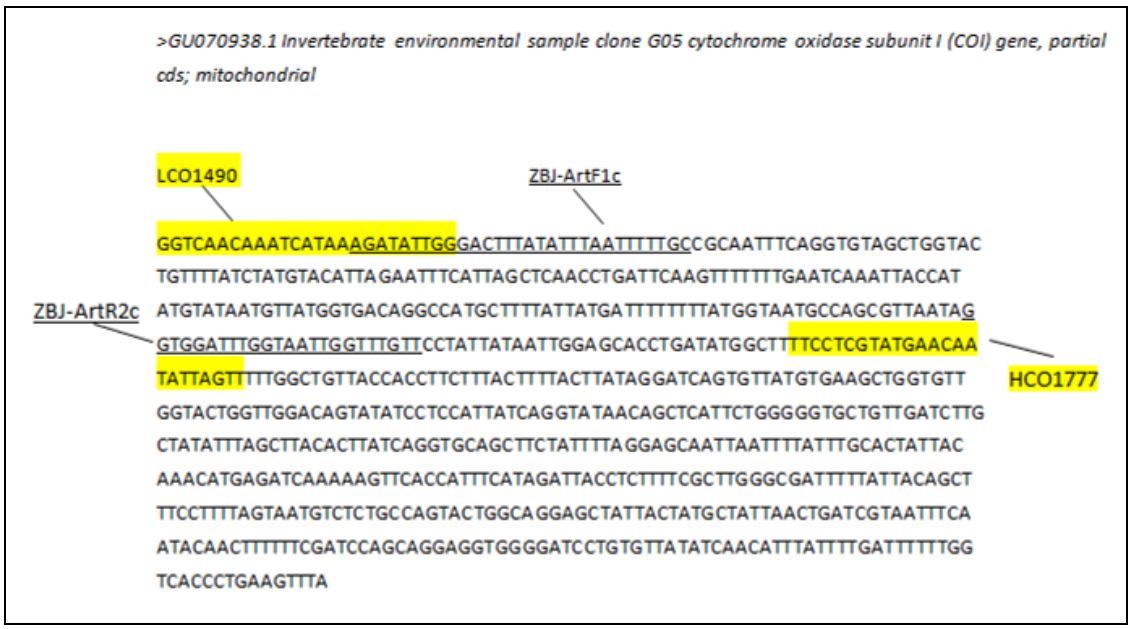


Figure 4. Alignment of database COI mitochondrial DNA sequence (GU070938.1 Invertebrate environmental sample clone G05 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial) showing complements of primers HCO1777 (Brown *et al.* 2012), LCO1490 (Folmer *et al.* 1994), ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al.* 2011) and their location.

DNA amplification

PCR for amplification of avian COI DNA from faecal samples took place in total volume of 20 µl and contained final concentrations of 1x GoTaq Green Flexi-buffer, 2 mM MgCl2, 250 µM each dNTP and 600 nM each of BirdF1 and AvMiR1 primers; plus 0.5 mg Bovine Serum Albumen (BSA), 0.667 U GoTaq G2 Flexi DNA Polymerase and 4 µL (i.e. 4-40 ng) of template DNA. The reaction programme comprised an initial denaturing step of 94°C for 5 min, then 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 45 s; and final extension at 72°C and a hold at 4°C. Purified PCR products from this reaction were Sanger sequenced externally (Genewiz, Takeley UK) using the forward primer BirdF1.

PCR for amplification of invertebrate DNA from remains in avian faeces took place in total volume of 20 µl at each of the two steps. The first PCR step contained final concentrations of 1x GoTaq Flexi buffer, 4 mM MgCl2, 50 µM each dNTP and 100 nM each of LCO1490 and HCO1777 primers; plus 2 mg BSA, 0.625 U GoTaq G2 Flexi DNA Polymerase, and 2 µL of template DNA. The first programme PCR comprised a denaturing step of 94°C for 150 s, followed by 25 cycles of 94°C for 30 s, 44°C for 30 s, 72°C for 45 s; and final extension at 72°C for 10 min and a hold at 4°C. The second PCR reaction contained final concentrations 1x GoTaq Green Flexi-buffer, 2.5 mM MgCl2, 50 µM each dNTP, and 400 nM each of ZBJ-ArtF1c and ZBJ-ArtR2c primers; plus 0.625 U GoTaq G2 Flexi DNA Polymerase and 2 µL of the completed first reaction mix. The second programme comprised an initial denaturing step of 95°C for 5 min, then 25 cycles of 95°C for 30 s, 52°C for 30 s, 72°C for 30 s; and final extension at 72°C for 7 min and a hold at 4°C.

PCR products were visualised on 1.5% agarose gels and were purified using the QIAquick gel extraction kit (Qiagen, Hilden, Germany), following manufacturers' instructions with the following alterations: PCR reactions showing a single band of amplified DNA molecule length were purified directly from the remaining reaction volume; by addition of 3 volumes Buffer QG, and 1 volume isopropanol per reaction volume, transferring this to a QIAquick spin column and proceeding from manufacturers' step 8. DNA was eluted from the

QIAquick spin column in 10 µl Buffer EB at 95°C, with 2 min incubation at room temperature before centrifugation.

Ligation of invertebrate COI and bacterial transformation/cloning

Purified invertebrate two-step PCR products were ligated to pGEM-t Easy Vector (Promega, Madison, USA), following manufacturers' instructions for sticky-ended ligation. Ligation was carried out overnight (17 h) in total reaction volume 5 µl with 1x Rapid Ligation Buffer, 25 ng pGEM-t Easy Vector, 2 U T4 DNA Ligase and 1.5 µl (45 - 60 ng) template DNA. Transformed vectors were inserted into competent DH5α *Escherichia coli* cells by heat shock transformation following pGEM-t Easy Vector manufacturers' instructions.

Colony PCR

For each faecal sample, 14 recombinant colonies on indicator plates were identified by blue/white screening, picked and transferred to PCR reaction of total volume 20 µL per colony. Final concentrations in reaction were 1 x GoTaq Green Flexi buffer, 2.5 mM MgCl2, 50 µM each dNTP, and 200 nM each of T7-long and Sp6-long primers; plus 0.625 U GoTaq G2 Flexi DNA polymerase. The PCR programme comprised an initial denaturing step of 94°C for 10 min; and 30 cycles of 92°C for 40 s, 50°C for 45 s, 72°C for 30 s and final extension 72°C for 10 min. From 14 colony PCR products, 10 were selected for purification and sequencing. 400bp were PCR products were purified using QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) by the above purification method, with the exception that DNA was eluted in 20µl of Buffer EB. Mean DNA concentration after colony PCR and purification was 75.6 ±17.2 ng µL-1. Purified samples were Sanger sequenced externally (Genewiz, Takeley UK) against the colony PCR forward primer T7-long.

Sanger sequence handling and species identification

Returned DNA nucleotide sequences included a length of sequence at both ends originating from the vector plasmid. This is an unavoidable result of i) sequencing cloned inserts to a bacterial plasmid and ii) using T7 from final PCR

of cloned plasmids as the sequencing primer to capture the full length of cloned insert DNA from prey. These vector regions were to be removed ahead of entering sequences to DNA barcode database searches. Trace files showing the chromatogram from automated sequencing of DNA were visually inspected in BioEdit 7.0.5.3 (Hall 1999). All sequences showed a transition between signal peaks that were poorly defined for the first c. 20 base pairs, to those that were clear and distinct (indicating more reliable base identification). This transition consistently took place upstream of the DNA region of interest, i.e. the cloned insert sequence originating from invertebrate prey. Nucleotide sequences were therefore considered suitable in quality for use as query sequences and for identifying potential prey taxa in this study.

Inspection of the vector plasmid sequence (Promega 2018) on each strand showed the vector sequence approximated 5'-GGCGGCCGCGGG AATTCGATT-3' on the sense strand, and 5'-CGGCCGCGAATTCACTA GTGAT-3' on the antisense strand, immediately before the cloned insert region. These were entered as recognition sequences for vector removal in software designed for manual editing of nucleotide sequences; DNA Baser 4.36.0.2 (Heracle Biosoft 2013). All raw returned sequences were collated in FASTA format and put through batch sequence cleaning in DNA Baser, specifying removal of the above recognition sequences as well as all upstream vector sequence. The output sequences were then aligned to visually assess success in vector removal. This also aided in manual cutting of vector from the minority of sequences not successfully cleaned by the software – commonly due to sequencing error over the vector region resulting in poor match to recognition sequence.

For assigning candidate prey taxa to original faecal samples, cleaned sequences were entered as queries to the Barcode of Life Database (BOLD) version 4 (http://www.boldsystems.org, Ratnasingham and Hebert 2007). To reduce redundancy in the query dataset, the bioinformatics software Geneious 11.1.4 (http://www.geneious.com, Kearse *et al.* 2012) was used to search for sequences with identical residues, and output only unique sequences (which retained their sample same and replicate number). The full set of study sequences was separately entered into nucleotide sequence clustering using

the online tool CD-HIT-EST (Li and Godzik 2006, Ying *et al.* 2010). A similarity threshold of 100% was specified. This identified which sequences were duplicates of the unique sequences carried forward to the database search. Sequences were entered in batches of c. 90 in FASTA format as queries to the species level barcode records database. BOLD searches do not automatically detect reverse-complement matches to query sequences, therefore batches of the reverse complement of each query sequence were also derived (http://reverse-complement.com/) and submitted with identical search specifications. 98% similarity between query and database sequences was required for a 'species-level' taxon to be considered a candidate organism of origin for dietary DNA in this study (following King *et al.* (2015)). For each query sequence returning matches, I inspected the top 99 matching sequences and recorded all database taxa and percentage similarity in sequence above 98%, up to a maximum of ten distinct species-like taxonomic units. Subspecies taxonomy was therefore counted as one taxon, while a fully named species and an unidentified congeneric species (e.g. '*Dilophus febrilis*' and '*Dilophus sp.*') were counted as two taxa. The global occurrence of each taxon was checked with web searches for distribution information, and taxa not known to have been recorded in Britain were flagged (following King *et al.* (2015)). If all matches about 98% similarity had no UK records, the first higher taxonomic level to contain species of UK occurrence was recorded as th taxon identity. Having established taxonomic candidate matches for each unique query sequence, the appropriate matches were subsequently assigned to all other sequences returned from samples in the current study, using the clusters of sequences identified in CD-HIT-EST analysis above.

Protocol for preparing bird faeces for Illumina MISEQ sequencing

DNA extraction

For next generation sequencing based analysis for avian diet, there was a requirement for concentration of template DNA entering initial steps to consistently exceed 10 ng/µl. Kit-based extraction from samples ahead of Sanger sequencing had given mean concentrations below this threshold, likely in part due to loss of DNA into solution during storage which was then lost

during decanting of ethanol from samples. For DNA extraction ahead of Next-Generation sequencing I freeze-dried samples and adopted a precipitation and re-suspension based technique as previously used with degraded DNA by D. Chaput (unpublished data, 2017).

Samples were freeze-dried to remove ethanol and stored and processed in 2 ml screw-top tubes. To begin lysis, lysis buffer at room temperature was added and mixed by vortex. Samples were re-frozen to -80 °C for at least 60 min to lyse cells and then briefly thawed in a 37 °C waterbath. Ceramic beads were added and samples were shaken at 30 Hz for 40 s using a TissueLyser II (Qiagen, Hilden Germany). To the 19-parts sample solution, 1 part SDS solution (10% w/v) and 0.1 parts proteinase K were added, and samples incubated for 1 h in a shaking incubator at 55 °C to continue lysis and protein digestion. Sample temperature was raised to 65 °C and to the 5 part sample solution, 1 part of 5 M NaCl solution was added and mixed by inversion, then 0.8 parts warm CTAB solution (10% w/v) added and mixed. Samples were incubated at 65 °C for a further 10 min. Samples were removed to room temperature, and then while chilled on ice were subject to two extractions of DNA by chloroform:isoamyl alcohol (chl:iaa, 24:1) wash. In extraction 1, 1 part of chl:iaa solution was added to 1 part of sample solution and mixed briefly by vortex. The mixture was centrifuged for 5 min at 14 000 x g at 4 °C and the top aqueous layer removed and retained on ice as the first extract. Noting volume of aqueous solution removed, this volume of chl:iaa solution was added to the original sample solution and briefly vortexed. The mixture was again centrifuged for 5 min at 14 000 x g at 4 °C, and the resultant top aqueous layer removed and retained on ice as second extract. To all tubes of extracts, 1 µl of linear polyacrylamide solution (LPA) was added to increase precipitation of DNA. To the 1 part volume of aqueous layer originally extracted, 0.7 parts of isopropanol was added and mixed by repeated inversion. Samples were then incubated in the dark at room temperature for at least 2 h to allow precipitation of DNA. Centrifugation at 4 °C took place at maximum speed over 30 min to pellet DNA, and all supernatant was removed leaving only the pellet of DNA and isopropanol precipitate. Pelleted DNA and tube walls were washed in ice-cold 70% ethanol and centrifuged at maximum speed for 10 min. Ethanol was

removed and the pellet air-dried for 5 min, before being resuspended in 50 µl of molecular grade water and mixed by flicking to minimise DNA degradation.

Illumina MISEQ sequence handling and species identification

In this segment, an amplicon sequence variants (ASVs) method using the R package Dada2 (Callahan *et al.* 2016) was used to collate and aid in identification of sequences read during the Illumina MISEQ run. Ahead of forwarding into Dada2, read sequences (hereafter 'reads') were grouped to correspond to their original samples by demultiplexing the raw data. This was achieved using the unique tag sequences annealed to primers (and therefore the amplicons) to determine sample of origin. In Dada2, read quality could be examined visually. This package was also used to filter/trim, to dereplicate and to merge reads. The first 30 bp and first 24 bp were removed from the forward and reverse reads of sequences, respectively, to remove the primer sequences from the read. Reads were truncated at 200 bp (200 was set as both the upper and lower limit), to further reduce reads to those of appropriate length to the present search of database COI sequences. Sequences were truncated if the read-quality of a base dropped to Q35 or lower. All reads with ambiguous bases were removed, and reads with more than two expected errors were also removed. Dereplication of reads in Dada2 is distinct to clustering of unique sequences, as carried out in the above Sanger sequencing workflow, in that both abundance and quality information are retained for each sequence. Each ASV was entered as a query to a search of all species-level barcodes in the Barcode of Life Database (BOLD) version 4 (Ratnasingham & Hebert 2007). Species barcode sequences were considered matches if similarity exceeded 97% (following Stackebrandt & Goebel 1994). The species with the highest similarity above this threshold was recorded, following King *et al.* (2015)

References

Balmer, D., Gillings, S., Caffrey, B., Swann, B., Downie, I., and Fuller, F. (2013) *Bird Atlas 2007–11.* Thetford: BTO.

Beavan, S. D., and Lock, M. eds. (2016) *Devon Bird Atlas: 2007-2013*. Devon Birds.

Brown, D. S., Jarman, S. N., and Symondson, W. O. C. (2012) Pyrosequencing of prey DNA in reptile faeces: analysis of earthworm consumption by slow worms. *Molecular Ecology Resources* 12(2): 259–266.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13: 581–583.

Callahan, B. J., McMurdie, P. J., and Holmes, S. P. (2017) Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal* 11: 2639–2643.

Chaput, D. unpublished (2017) *CTAB / EDTA DNA extraction protocol for filters*. BBSRC/DBT/Newton Aquaculture Project, Exeter.

Douglas, D. J. T., Evans, D. M., and Redpath, S. M. (2008) Selection of foraging habitat and nestling diet by Meadow Pipits Anthus pratensis breeding on intensively grazed moorland. *Bird Study* 55(3): 290–296.

Dartmoor Upland Bird Nest Group (/2017) About - Dartmoor Upland Bird Nest Group. *Dartmoornests.Org*. Accessed: 22nd July 2019 <http://www. dartmoornests.org/about.html. >.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology* 3: 294–299.

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic Acids Symposium Series No. 41.* Oxford: Oxford University Press.

Hebert, P. D. N., Cywinska, A., Ball, S. L., DeWaard, J. R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B - Biological Sciences* 270: 313–321.

Hebert, P. D., Stoeckle, M. Y., Zemlak, T. S., and Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS biology* 2(10): 1657-1663.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., and Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1647-1649.

Kerr, K.C., Lijtmaer, D.A., Barreira, A.S., Hebert, P.D. and Tubaro, P.L., (2009). Probing evolutionary patterns in Neotropical birds through DNA barcodes. *PLoS One* 4 (2): p.e4379.

King, R. A., Symondson, W. O. C. and Thomas, R. J. (2015) 'Molecular analysis of faecal samples from birds to identify potential crop pests and useful biocontrol agents in natural areas', *Bulletin of Entomological Research* 105 (3): 261–272

Lijtmaer, D. A., Kerr, K. C., Stoeckle, M. Y., and Tubaro, P. L. (2012) DNA barcoding birds: from field collection to data analysis. In *DNA Barcodes*. Totowa, NJ: Humana Press.

Noakes, P. (2013) 'Catching cuckoos', *BTO Ringing News*, 13(1): 11–11.

Promega (2018) *pGEM® and pGEM® -T Easy Vector Systems Technical Manual #TM042*. Promega Corporation, Madison. Available at https://www.promega.com/-/media/files/resources/protocols/technical-manuals/0/pgem-t-and-pgem-t-easy-vector-systems-protocol.pdf

Ratnasingham, S., and Hebert, P. D. N. (2007) BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* 7(3): 355–364.

Stackebrandt, E., and Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology* 44(4): 846-849.

Zeale, M. R. K., Butlin, R. K., Barker, G. L. A., Lees, D. C., and Jones, G. (2011) Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources* 11(2): 236–244.

3. Vegetation correlates of nestling condition and provisioning of meadow pipits *Anthus pratensis*, a cuckoo host in semi-natural grassland

Abstract

The meadow pipit *Anthus pratensis* is a globally near-threatened passerine and frequent host of the parasitic common cuckoo *Cuculus canorus* in Britain. The cuckoo is declining in Britain but shows relatively positive population trends in Scotland and semi-natural grass or heathland. In both cases, open semi-natural land is the predominant habitat and meadow pipits are the primary host. To begin disentangling whether the relative positive trends in cuckoo relate to habitat, or quality of the meadow pipit host, relationships between meadow pipit breeding and semi-natural habitat variables must be studied; followed by study of how these relationships differ if the nest is parasitised by cuckoo. While the former has been previously studied there has been no research of the latter, in any host. Here, the habitat associations of meadow pipit foraging, brood size, nestling 'body condition' (nestling mass given tarsus length), and provisioning behaviour (hourly nest visit rate and prey load size) were examined in sympatry with a study population of cuckoos which parasitises pipit nests on Dartmoor, UK. We monitored non-parasitised meadow pipit nests, measured nestling mass and tarsus length, and observed adult provisioning behaviour, following the methodology of previous meadow pipit studies. Cover of vegetation in 50 x 50 m squares in the 'nest territory' (defined as 100 m radius around nests), and 10 x 10 m squares where foraging occurred, was quantified. Pipits mainly foraged within 100 m of the nest, and showed preference for 50 m squares with vegetation height heterogeneity including grazed pastoral grass, complex low vegetation, and trees. Within 50 m squares in the nest territory, pipits selected 10 m squares with relatively extensive cover of grazed grass, and lower cover of taller vegetation and bare substrate. Nestling body condition did not significantly vary with territory vegetation cover across study areas, but correlated positively with cover of main nesting vegetation at the primary study site. Provisioning visit rate and prey load size negatively correlated with bracken cover, visit rate negatively correlated with tall meadow grass cover, and prey

load size negatively correlated with cover of dead or burnt gorse. Daily nest survival did not significantly vary with nest provisioning variables. Correlates identified here largely concur with meadow pipit studies in other habitats such as upland *Molinia caerulea* grassland, lowland agricultural grassland and grazed saltmarsh; but also indicate selection of edge habitat between moorland and pastoral grassland by foraging pipits on Dartmoor, and that bracken and burnt gorse may negatively impact on foraging efficiency of nesting meadow pipits, with implications for upland management.

Introduction

Long-term variation in nesting success is often a key demographic driver of population change in birds (e.g. Douglas *et al.* 2014). For species rearing altricial young, success involves adults sheltering and provisioning chicks to reach sufficient body condition to leave the nest, whilst avoiding predation. Provisioning behaviour therefore has a critical role in nesting success (e.g. Boersma & Rebstock 2009). Provisioning is linked to habitat via birds' nesting and foraging decisions, and via the habitat and food-plant preferences of prey. Through this string of associations, habitat change in foraging or nesting areas – such as land-use or land-use change – can be a powerful driver of population change. The meadow pipit *Anthus pratensis* is a passerine bird of international near-threatened status (IUCN 2017). The UK is of high importance, accounting for roughly 17% of the global population, specifically in UK upland habitats which appear to represent a refuge from significant land-use change in other suitable open habitats in the lowlands (Pearce-Higgins *et al.* 2009).

Decreases in meadow pipit clutch and brood size, and fledglings per breeding attempt, have been recorded by UK nest monitoring since 1967 (British Trust for Ornithology (BTO) Nest Record Scheme NRS, 1939-present); as has an increase in egg-stage failure rate (Massimino *et al.* 2017). Distribution of confirmed breeding has contracted nationally but particularly in lowland agricultural areas (Balmer *et al.* 2013, c.f. Sharrock 1976). The meadow pipit has historically been a key host of the parasitic common cuckoo *Cuculus canorus* in UK semi-natural grasslands (Glue & Morgan 1972, Brooke & Davies

1987). These grasslands are a relative stronghold habitat for the cuckoo whose populations have declined significantly in most previously frequented habitats (Massimino *et al.* 2017). Cuckoos have also significantly increased in Scotland since 1995 despite overall UK declines (Harris *et al.* 2018), and here the main habitat and host are similarly semi-natural grass and heathland and meadow pipits, respectively. Cuckoos deposit single eggs into host nests (Chance 1922), where the nestling is raised by the 'foster' parents mistakenly as their own young. While habitat use and prey selection of fledgling cuckoos increasingly resemble those of adult cuckoos after independence from the host foster parents (Wyllie 1981), the ecology of nestlings is closely associated with that of the provisioning host; and related to adult cuckoo ecology only by the habitat and nest site in which the egg is initially laid. The cuckoo is a long-distance migrant, potentially vulnerable to many factors such as changes in climate, weather and habitat that could impact on prey resources at many stages of the migration route (Hewson *et al.* 2016, Thorup *et al.* 2017, Jacobsen *et al.* 2017). However, the present study focuses on potential driving factors on the cuckoo breeding grounds, specifically habitat and host meadow pipit breeding factors. Even before fledging there is evidence that food provisioning of cuckoo nestlings by different host species causes variation in fledgling mass (Kleven *et al.* 1999) that could cause host-specific variation in cuckoo juvenile survival rates during migration from the natal area. Furthermore, host species such as the meadow pipit have themselves undergone breeding range contractions that reflect changes in agricultural land-use which have i) reduced safety and suitability of vegetation structure for nesting and ii) caused loss of invertebrate prey for adults and nestlings (Vickery *et al.* 2001). Nestling cuckoos are vulnerable to these same land use changes as they are dependent on provisioning from insectivorous birds (Wyllie 1981).

The meadow pipit's predominance as the host species in a UK stronghold habitat for the cuckoo means it is urgent to study how nest-stage demography and behaviour of meadow pipits relates to land use, in order to extricate potential habitat roles from host roles in driving cuckoo population change. Proving direct links between fine-scale habitat factors and nest success is difficult as adult quality is likely to also vary with habitat or fine-scale location

within a site (Davies *et al.* 2014). Habitat preference is itself scale-dependent, therefore simultaneous study at multiple scales is necessary (Vanhinsberg & Chamberlain 2001, Vickery & Arlettaz 2012, Šálek *et al.* 2016). Further to observing general habitat use, many studies also link this to nest biology by observing rate of provisioning visits to the nest. However, provisioning is likely to additionally vary through size, number, taxon (and therefore nutritional quality) of prey delivered, or distance flown to collect food; none of which is captured by measuring visit rate alone. Provisioning is therefore best captured by measuring visit rate, details of prey load size (and taxon) provisioned, foraging habitat selection and distance. When studying invertebrate diet of nestlings, differing study techniques have been shown to generate different estimates of prey composition (Kleintjes & Dahlsten 1992, Moreby & Stoate 2000, Grim & Honza 2001). Analysis via sequencing of prey DNA present in faeces or stomach contents may carry less detection bias towards hard-bodied prey than microscopy, and less bias towards large prey than video/direct observation. DNA base sequence of the mitochondrial gene COI (cytochrome-b oxidase I, *cox1*) is generally consistent within, and variable between, animal taxa (often at the level of species), meaning molecular approaches may also allow identification of prey to finer taxonomic level. Nutritional value of invertebrate taxa has been most studied in context of commercially reared insects for animal husbandry and taxa relevant to human consumption (Soler *et al.* 2008), but more recently the nutrient quality of taxa taken by passerine birds has been studied in the natural environment (e.g. Razeng and Watson 2014). While higher fraction of chitin content is cited to constitute lower quality prey, as this material is often regurgitated in pellets (Soler *et al.* 2008), this conclusion disregards the trade-off consumers would be likely to exercise if high chitin content co-occurs with high nutritional value of digested parts, or with larger body size, for example in Coleoptera and Orthoptera.

The present study investigated what types and structure of vegetation were selected for foraging by breeding meadow pipits provisioning nestlings; and how nestling condition and adult provisioning behaviour varied with nesting and foraging habitat, respectively. It also tested whether daily nest survival rate related to adult nest provisioning rate measures. The study followed previous

research on meadow pipits (Evans *et al.* 2006, Douglas *et al.* 2008, Vandenberghe *et al.* 2009, van Klink *et al.* 2014). However the present study differed from past studies in its use of multiple nest provisioning variables to capture variation in provisioning behaviour, and its objective of expanding many of its analyses to include nests parasitised by a sympatric study population of common cuckoo, and test whether host adults at parasitised nests show indication of different habitat use or nestling prey selection.

Previous studies of habitat associations, diet and nesting of the Meadow pipit in the UK have focused on 'upland' (>200m altitude) grass and heathland populations (Vanhinsberg & Chamberlain 2001, Douglas *et al.* 2008, Vandenberghe *et al.* 2009), where pipits remain a key cuckoo host species. Pipit populations in UK lowland and coastal grassland, where the species formerly accounted for around half of cuckoo hosts (Glue & Morgan 1972), have declined, and there has been significant loss of cuckoo from lowland and coastal Bird Atlas squares (Sharrock 1976, Gibbons *et al.* 1993, Balmer *et al.* 2013). Meadow pipits in these habitats have received limited research effort in the UK (Wilson *et al.* 1996, Vickery *et al.* 2001, Atkinson *et al.* 2005), and in mainland Europe (Kosicki & Chylarecki 2013, van Klink *et al.* 2014).

Meadow pipits are indicated to favour low vegetation height (<30 cm) when foraging in upland and lowland grassland (Vickery *et al.* 2001, Atkinson *et al.* 2005, Douglas *et al.* 2008, Vandenberghe *et al.* 2009). There is little evidence of a preferred vegetation density at a given sward height (reviewed Pearce-Higgins *et al.* 2009). Pipits breeding in uplands showed highest abundance in a (site-level) mosaic of vegetation types including heather, grassland and bog (Vanhinsberg & Chamberlain 2001, Pearce-Higgins *et al.* 2009). Vegetation heterogeneity is also a recommended management goal for lowland grasslands to support meadow pipit foraging and breeding (Vickery *et al.* 2001, Atkinson *et al.* 2005).

Nestling diet has been previously studied either from bill contents of provisioning adults (e.g. Walton 1979, Douglas *et al.* 2008) or analysis of nestling faeces (e.g. van Klink *et al.* 2014). Prey taxa of importance to meadow pipit nestlings reported across a range of breeding habitats are larvae of insect

order Lepidoptera and of the dipteran family Tipulidae; and adult spiders (Walton 1979, Wilson *et al.* 1996, Evans *et al.* 2005, Douglas *et al.* 2008, Hågvar *et al.* 2009, van Klink *et al.* 2014). These taxa are among the most ubiquitous prey reported across moorland birds in general (Buchanan *et al.* 2006). While studies of meadow pipits have varied in the detail to which they have identified prey, they have largely noted a broad diversity of prey (Walton 1979, Cramp 1988), with the most frequent taxa often reflecting largest taxa of greatest abundance in the study area (van Klink *et al.* 2014). In provisioning observations, most foraging for nestling prey took place within 100m of the nest (Douglas *et al.* 2008, Vandenberghe *et al.* 2009, van Klink *et al.* 2014). Provisioning distance and habitat use have also been studied in the two other main cuckoo hosts in Britain, the reed warbler *Acrocephalus scirpaceus* (foraging chiefly within 50m of the nest; Bibby and Thomas 1985) and dunnock *Prunella modularis* (maximum 60m from the nest; Bishton 2001).

In the present study the objective was to test the relationships between meadow pipit breeding factors and semi-natural habitat factors, within a study area where common cuckoos parasitising meadow pipits were also receiving field study. This preceded analyses reported in chapter 4 that were focused on one study site at which additional nests parasitised by cuckoos were located and studied. It was predicted that meadow pipits would show selection towards habitat with high invertebrate abundance and fine-scale patches where invertebrates were most detectable and accessible. Cover of habitat associated with high abundance or accessibility of invertebrates, within 100 m of the nest, was furthermore predicted to have a significant positive correlation with nestling body condition, and cover of vegetation with poor accessibility for foraging such as dense dwarf-shrub cover and wet bog patches was expected to negatively correlate with nestling condition. Cover of dense dwarf-shrub, woody vegetation and bog on foraging plots was predicted to negatively correlate with provisioning visit rate and potentially prey load size, by impacting on search and capture times in foraging meadow pipits. Predictions were not made for correlation between provisioning rates and nesting success due to the expected trade-offs between provisioning rate variables, and between parental energy expenditure and nestling growth. The study asked the following questions: i) Do

meadow pipits in the study area show selection among the recorded vegetation types at the (i) 50 m and (ii) 10 m scale within 100 m of the nest, when foraging for prey to provision to young? (iii) Does meadow pipit brood size or nestling condition vary with nesting habitat vegetation? (iv) Are nest provisioning rate or prey load size dependent on foraging habitat vegetation? (v) Does nestling-stage daily nest survival rate show a relationship with provisioning rate, prey load size or foraging distance?

Methods

Study areas and nest finding

Nests of meadow pipits were located at two study areas in Dartmoor National Park, UK in April to July in 2015, 2016 and 2017. Holne Moor (50° 31' 20" N, 03° 51' 43" W) is a large semi-natural grassland area of 300-400 m altitude within the main grassland extent of the Park, bordered to the east by pastoral grassland agriculture and to the north by native ancient oak *Quercus* and birch *Betula* woodland. Meldon Common (50° 39' 40" N, 03° 50' 52" W) is a smaller semi-natural grassland area separate from the main moor, of 300-400 m altitude, bordered on all sides by pastoral grassland agriculture (Fig. 1). Both sites receive mixed grazing during the bird breeding season in April to July; at Holne Moor from sheep, cattle and ponies, and on Meldon Common from ponies. Most trees in the semi-natural grassland were rowan *Sorbus aucuparia* and hawthorn *Crataegus monogyna*.

Nests were found by extensively searching each study site on foot and watching pipits carrying food back to young (or incubating females). Female pipits were watched back to nests with eggs. A few nests were found by chance flushing of the female while passing. Most nests were found at the nestling stage. Nestling mass and size (tarsus length) biometrics were recorded in 2015 and 2016. Observations of provisioning behaviour were carried out at Holne Moor 2016 and 2017, and 2016 only at Meldon Common.

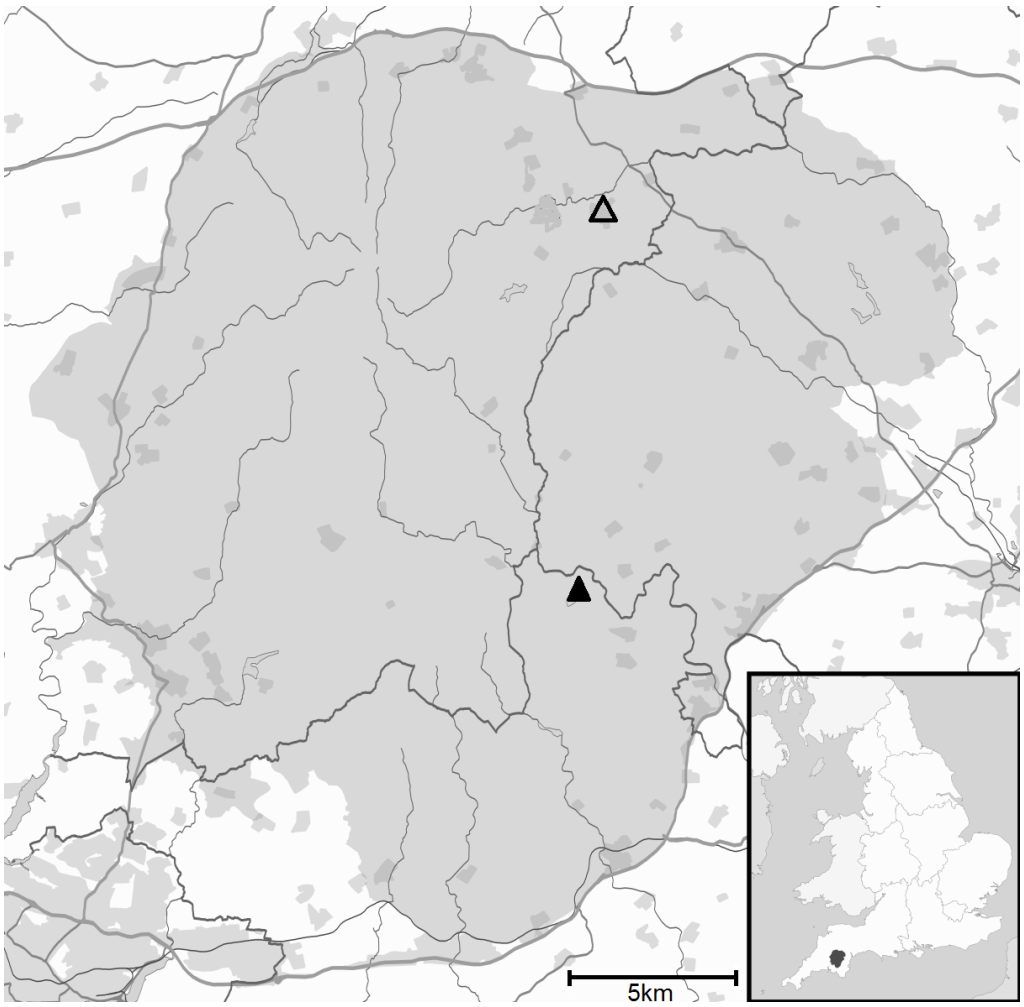


Figure 1. Location of study areas Holne Moor (filled symbol) and Meldon Common (open symbol) in Dartmoor National Park. Inset, location of Dartmoor in southwest Britain.

Nest monitoring and faecal sampling

Visits to monitor nest contents were carried out every 2-4 days, from the date the nest was found until outcome (success or failure) was established. Nest visits were avoided in cold and wet weather to minimise effects of study on nestling development. Pipit nestlings were handled for up to two minutes on up to three occasions between ages 3 and 10 days, under Natural England license (two handlings) or BTO Ringing Scheme license (one handling for ringing). In 2015 and 2016, nestling mass was measured to 0.25 g with a 30 g spring balance (accuracy ±0.3%) (Pesola, Feusisberg, Switzerland); and the tarsus

length was measured to 0.1 mm with callipers (Wiha, Schonach im Schwarzwald, Germany).

Nest observations

Observations of parental provisioning behaviour were carried out during the nestling stage, at a distance allowing both a view of the wider foraging area and a telescope view of the size of food carried by adult birds to the nest (range 36 m to >200 m). Time of visit by a parent bird to the brood, plus prey load size as a proportion of bill length, were recorded. Arriving at nest sites with food, adult pipits often landed at a distance from the nest itself, out of the observer's view. Time of entering the nest was difficult to determine but birds typically flew directly from the nest after provisioning. To more accurately measure period between feeds, time of visit was therefore defined as the time at which the adult flew from the nest. Locations used for foraging during provisioning were recorded with handheld GPS (Garmin, Lenexa, USA; accuracy ±3 m in study area) immediately after the observation session ended. Observation sessions were carried out at the same hour of day for a given nest, ranging from 0700 to 1900 BST. Observation sessions started after alarm calling caused by observer arrival ceased (as in Douglas *et al.* 2008), and ran for 60 min or until five spatially distinct foraging locations were identified; whichever was soonest. Foraging locations considered distinct from each other were at least 5 m apart. The shortest observation session on this basis was 22 min. Nests located at earlier stages (eggs, small young) were prioritised for faecal sampling and observation over those found at later stages, to increase opportunity for repeat sampling and observation sessions throughout nestling development. While the number of observation sessions of a nest was not limited, ultimately the maximum number of sessions for a nest was four. For each observation session the hourly rate of feeding visits was calculated as: rate = 60 / t , where t was the mean period in minutes between observed feed visits during the session. This was adopted as birds were assumed to be disturbed from their normal provisioning rate for a variable period at the start of observation sessions, even after alarm calling ceased.

Table 1. Vegetation and other habitat variables measured in both 50 m squares around nests (Fig. 2) and 10 m squares used for foraging. All variables except for presence variables were measured on an adapted Braun-Blanquet scale. Images of vegetation types in Figure 3.

Variable	Code
Low gorse *Ulex spp.* (<75 cm)	LG
Medium gorse (75-150 cm)	MG
Mixed gorse and tufted grass (<75cm)	GG
Low homogeneous grasses (<10 cm)	GR
Grazed pastoral grass	PG
Long homogeneous-height grasses, i.e. hay meadow (>10 cm)	HO
Tufted long grasses >10 cm	TU
Dead/burnt gorse stalks and short grass <10 cm	DG
Live bracken	BR
Heather	HE
Bilberry	BI
Mosses	MO
Bare rock	RK
Bare ground/earth	BGRD
Juncus	JU
Bog	BO
Open water (still or flowing)	OW
Shrubs ≥ 1.5 m high	SHRUB
Trees ≥1.5 m high	TREE
Presence of shrubs ≥ 1.5 m high	PRESSHRUB
Presence of trees ≥1.5 m high	PRESTREES
Presence of trees ≥3 m high	PRESTREES3

Vegetation measurement

Vegetation in a 100 m radius around each nest, and in a 10 x 10 m square around each identified foraging location, was measured between 1 and 31 August to avoid disturbance to breeding birds. 10 x 10 m squares overlying foraging locations are hereafter referred to as foraging plots. The 100 m radius was identified as the distance within which most meadow pipit foraging takes place while provisioning (Douglas *et al.* 2008, Vandenberghe *et al.* 2009). Vegetation within 100 m was measured in 12 squares of 50 x 50 m (Fig. 2). Vegetation variables related to major vegetation and substrate types, some of which were split into height classes to capture habitat structure (Table 1) (Fig. 3). Vegetation cover was recorded using an adapted Braun-Blanquet scale of 0 to 5 representing percentage cover (0 = 0 %/absent; 1 = up to 5%, 2 = 5-25%; 3 = 25-50%; 4 = 50-75%, 5 = 75-100%) (Braun-Blanquet 1932). Field data collection treated each vegetation variable as a layer of up to 100% cover, with its value unaffected by presence of overlying layers (e.g. grasses and bracken overgrowing them may each score up to 5 (75-100%)). Presence or absence of gorse shrubs over 1.5 m high, trees over 1.5 m high, and trees over 3 m high, were also recorded.

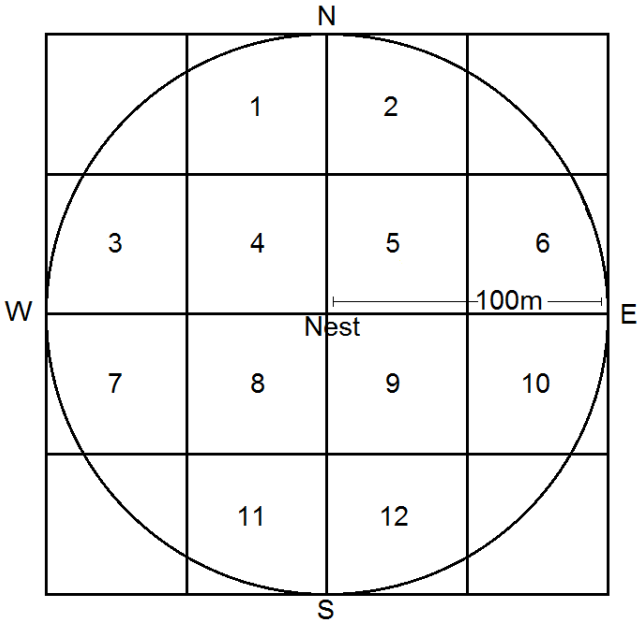


Figure 2. Orientation of 50 x 50 m habitat survey squares in 100 m radius of meadow pipit nests.

Data analysis

(i) Foraging habitat preference (50 m scale)

To test whether pipits foraging within 100 m of the nest showed selection among the recorded vegetation types, I modelled the relationship between vegetation (habitat) cover scores and the number of observed foraging visits to all 50 x 50 m squares (n = 12 per nest) within 100 m of all observed nests. For each habitat variable, the distribution of cover scores (0 to 5) were examined, and scores of low frequency (≤10 occurrences) were pooled with the adjacent score so that each score accounted for more than 10 surveyed 50 m squares. As scores were pooled I kept track of the range of percentage covers each aggregated score represented. I fitted a general linear model (GLM) with negative binomial error distribution to correct for potential over-dispersion of data (Crawley 2012). The final model was reached by forward selection from the null model. The correlation matrix of all potential model variables was first inspected, and all variable pairs or groups covarying with Pearson's correlation coefficient $r \geq 0.7$ magnitude (±) were highlighted for potential exclusion. Each variable in a pair or group was then tested alone (with nuisance variables and random factors as detailed below), and the variable of lowest probability value *P* was retained and all others excluded from model selection.

The null model included nest identity as a fixed factor to account for variation in observation effort between nests, and distance band in which the square lay (0-50 or 50-100 m) as a fixed factor. Forward selection proceeded from the null model containing these nuisance variables only, checking the type III output for the model including each habitat variable in turn, and at the end of each round selecting the habitat variable of lowest probability value for inclusion, until no further variables could be added at $P < 0.05$.

(ii) Foraging habitat preference (10m scale)

To test whether pipits foraging within 100 m of the nest showed fine-scale selection of vegetation types relative to their availability, the cover scores (0 to 5) within each identified 10 x 10 m foraging plot for each vegetation variable, were analysed alongside the associated cover score (0 to 5) of the vegetation

variable in the encompassing 50 x 50 m square of surveyed habitat. For each vegetation variable I used a paired Wilcoxon Signed Rank test (with continuity correction) to compare the scores at the two scales. Each test reported the summed rank *V* of positive differences in cover score for a vegetation variable. A higher value of *V* for a vegetation type signified greater percentage cover on 10 m foraging plots than in surrounding 50 m squares, lower values signified lower cover on foraging plots. These respectively indicated selection or avoidance of vegetation relative to availability. The modelling was carried out separately for foraging plots at Holne Moor and at Meldon Common, due to difference in presence of some habitat variables between study areas.

(iii) Habitat associations with brood size and nestling condition

To test whether brood size varied with nesting habitat, I modelled the relationship (at the replication level of nest) between maximum brood size and the cover of recorded vegetation types across the 12 surveyed 50 x 50 m squares within 100 m of each nest, including study area (Holne Moor, Meldon Common) as a fixed factor. Cover scores of each vegetation variable (and presence scores of presence variables) were pooled to a mean score across the 12 50 m squares surrounding the nest (Fig. 2), so that each nest had one mean value of each vegetation variable. Vegetation variables were included as covariates in a general linear model with normal error distribution, with brood size as the response variable. Model selection proceeded by backward selection from a 'full-model'. The correlation matrix of all potentially included variables was first inspected, and all variable pairs or groups covarying with Pearson's correlation coefficient $r \geq 0.7$ magnitude (±) were highlighted for potential exclusion. Each variable in a pair or group was then tested alone (with nuisance variable of study area (site) as detailed above) and the variable of lowest probability value *P* was retained and all others excluded from model selection. Backward selection then proceeded from the full model, consulting the type III (ANOVA) output for each successive model composition and deleting the variable of highest probability value, until all variables included in the model were significant at $P < 0.05$.

To test whether nestling condition varied with nesting habitat, I fitted a general linear mixed model (at the replication level of nest) with normal error distribution, using the response of mean nestling mass (across nestlings measured in the nest visit) to mean cover (across the 12 50 m squares) of each vegetation variable, while controlling for mean nestling tarsus length (across nestlings measured in the nest visit) and including nest identity as a random factor. Study area (Holne Moor, Meldon Common) was included as a fixed factor. The vegetation variables and brood size were included as covariates. Beginning with the full model, model variables were removed by a backward selection approach as described above, except for tarsus length which was retained throughout. The final model was reached when all included variables were significant at $P < 0.05$.

In order to test these relationships specifically for the site at which nestling cuckoos were also located and studied, both modelling runs were repeated with data for Holne Moor only.

Finally, in order to test whether nestling body condition varied with foraging habitat vegetation, I used general linear mixed models to test the relationship (at the replication level of nest) between nestling condition and mean cover of vegetation on used foraging plots. Using the complete list of all visits made by observed adult pipits to all identified foraging plots per nest, and the associated 10 x 10 m square habitat cover scores per foraging plot, a mean 'foraged' cover score for each vegetation variable was pooled per nest. Data was tabulated at the replication level of nestling measuring visit, with mean nestling mass (across nestlings measured in a nest visit) as the response variable and mean nestling tarsus length (across nestlings measured in a nest visit) as a covariate, plus brood size and mean 'foraged' cover score of vegetation per nest as covariates, and nest identity as a random factor. The full model was subject to backward selection as described above. Data entering this analysis was collected only in 2016, so sample size was relatively low.

(iv) Habitat associations of provisioning behaviour

To test how provisioning rates at non-parasitised meadow pipit nests varied with foraging habitat used, I used generalized linear mixed models to test (at the

level of nest) how hourly provisioning visit rate (per observation session), and mean size of delivered prey loads (per observation session), varied with mean cover of vegetation across used foraging plots per observation session. Using the complete list of all visits made by observed meadow pipits to all identified foraging plots per observation session, a mean 'foraged' cover score for each vegetation variable was pooled per observation session. Hourly provisioning visit rate of pipits per observation session was calculated as rate = 60 / (mean period in mins between nest provisioning visits). Data was tabulated at the replication level of observation session. The response variables were, in turn, hourly provisioning visit rate and mean prey load size. Nest identity was included as a random factor. Mean foraging plot vegetation cover scores, observer vantage point distance (VPDIST), date, start hour (START_HR), duration, hatch date (renumbered with 01 May = 1), brood age in days (CHICKDAY) and brood size (NCHICK) were included as covariates, and observation session weather scores were included as fixed categorical factors (WIND, RAIN and CLOUD scored as per Breeding Bird Survey methodology, BTO 2018). Model selection proceeded by backwards selection as described for analysis (iii).

(v) Provisioning rates and nestling stage survival

To test whether nestling stage survival (of nests that had survived to point of receiving provisioning observations by the study) varied with provisioning visit rate, mean prey load size and mean foraging distance, I used a generalized linear mixed model with binomial distribution and logit link, to test the response FAIL denoting whether the nest ultimately failed (1) or successfully fledged (0), as determined from nest contents monitoring. I calculated the number of days the nest was monitored from the first nest check where nestlings were present, to when the nest failed or fledged, which if not known with certainty was the halfway point (to the nearest 0.5 days) between the last visit recording live nestlings and the first visit recording no live nestlings. From the tabulated data at the level of observation session I modelled effect on the response FAIL with nest identity as a random factor, log number of days monitored as an offset, and provisioning visit rate, mean prey load size and mean distance per observation session as covariates, proceeding by backward model selection as detailed in

a)

b)

c)

d)

e)

f)

g)

h)

i)

j) k)

l)

m)

n)

Figure 3. a) Low gorse LG with largely mixed low gorse and grass GG in background. b) A cluster of medium height-class gorse MG 75-150 cm tall. c) Mixed low gorse and grass GG in early August. d) Short semi-natural grassland grasses GR. e) Short pastoral grass PG (grazed by horses). f) Homogeneous height tall pastoral grasses HO, a hay meadow in late June (photo credit Adrian Platt, used under creative commons license). g) tufted structure semi-natural grassland grasses TU. h) burnt gorse stalks with short grass DG, with flowering new-growth gorse from more recent years LG. i) Bracken *Pteridium aquilinum* BR showing growth and volume in mid June (left) and July (right). j) Heather HE as found growing through gorse. k) bare ground BGRD. l) Close view of Bilberry BI growth on Dartmoor, here surrounding a meadow pipit nest entrance. m) *Juncus* rushes JU. n) Gorse shrub over 150 cm tall SHRUB/PRESSHRUB.

analysis (iii). I also separately tested the responses of FAIL to hatch date, and the interaction term between rate and mean prey load size, as covariates. Both first and second broods of meadow pipit were included in analyses. Hatch date was included as a covariate in some analyses to account for linear variation in hatch date. First vs follow-up broods were assumed not to specifically carry

differences in parental nesting or foraging habitat selection, provisioning rate, or daily nest survival, that were not captured by including hatch date. All statistical analyses were conducted using R version 3.5.0 software. Mixed modelling was carried out using the R package *lme4* (Bates *et al.* 2015) with probability values estimated using *lmerTest* (Kuznetsova *et al.* 2017).

Results

75 observation sessions totalling 67.3 h (57 sessions, 51.0 h, at Holne Moor. 18 sessions, 16.2 h, at Meldon Common) were completed for 41 meadow pipit nests (30 at Holne Moor, 11 at Meldon Common). 158 foraging plots were identified across 39 nests.

(i) Foraging habitat preference (50 m scale)

More than 75% of observed foraging took place within 100 m of nests. This was the case both overall (n = 395 foraging plot visits, quartile 1 (q_1) = 29 m, median = 48 m, q_3 = 83 m.) (Fig. 4) and at each site (Holne Moor: n = 302, q_1 = 20 m, median = 45 m, q_3 = 77 m. Meldon Common: n = 93, q_1 = 34 m, median = 50 m, q_3 = 83 m.). Foraging visit frequency to 50 x 50 m squares within 100 m of the nest (n = 468 squares across 39 nests) was higher in the 0-50 m distance band, and increased with presence of pastoral short grass (PG) (Fig. 3e) and trees (PRESTREES) There were significantly more foraging visits where cover of tufted semi-natural grassland grasses (TU) (Fig. 3g) was 50-75 %, and fewer visits where cover of medium (75-150 cm) gorse shrubs (MG) (Fig. 3b) was below 5% or above 25%. Foraging visits had a marginally significant positive association with presence of bare ground (Table 2). Two observed nests were excluded from habitat preference analysis. One was excluded because none of its 12 surveyed 50 m squares was visited for foraging by the adult pipits. One was excluded because the nest was within a few metres of an included nest, therefore its habitat data would cause replication in the dataset.

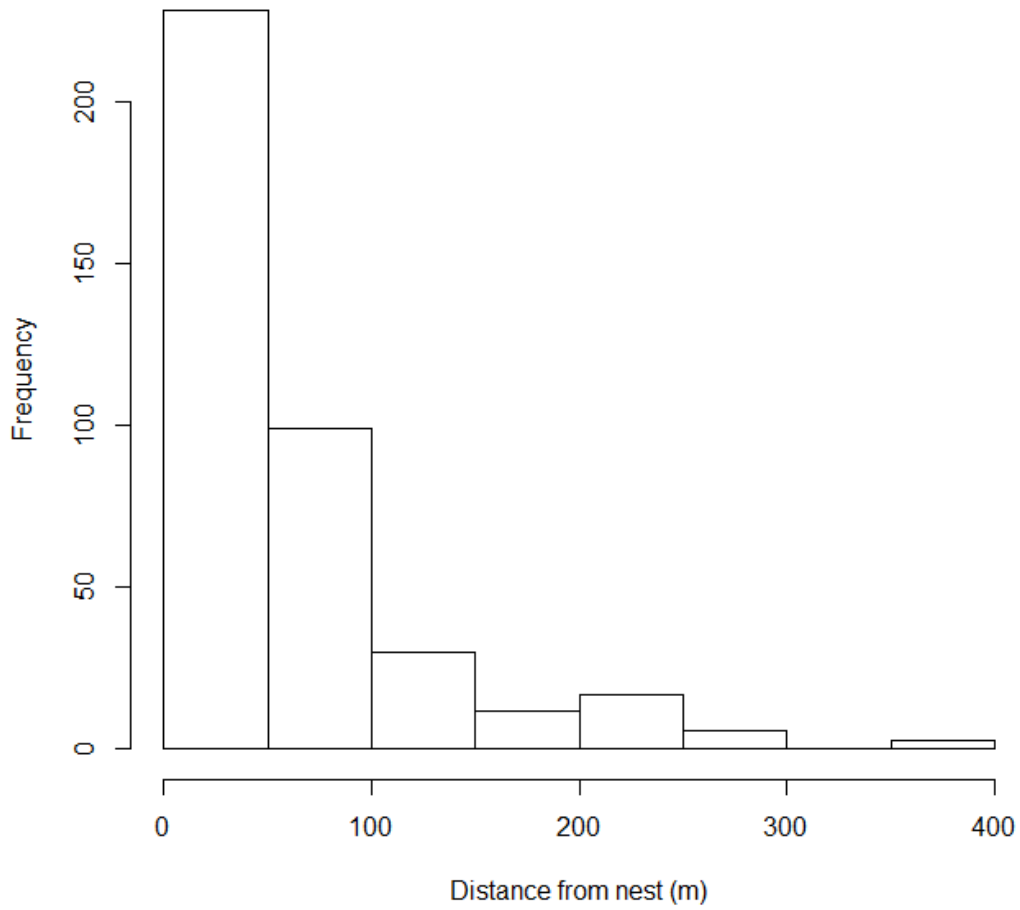


Figure 4. Frequency of foraging distances of provisioning meadow pipits across two study areas in Dartmoor National Park (all observed foraging trips, n = 395).

(ii) Foraging habitat preference (10 m scale)

At both sites, the identified 10 x 10 m foraging plots within 100 m of the nest had significantly greater cover of short semi-natural grassland grasses (GR) (Fig. 3d) than their enclosing 50 x 50 m square (paired Wilcoxon Signed Rank tests, n = 158, Holne Moor n = 126, sum of ranksof positive sign $V = 5484.5$, $P < 0.0001$, Meldon Common n = 32, V = 258.5, P < 0.05). At Meldon Common, foraging plots had less cover than their enclosing 50 x 50 m square of mixed and shrub gorse vegetation (MG, GG (Fig. 3c), DG (Fig. 3h) and SHRUB (Fig. 3n)); bracken (BR) (Fig. 3i), bilberry (BI) (Fig. 3l), bare rock (RK), and trees (TREE) (Table 3). These differences were also significant on Holne Moor except for bilberry; and additionally, foraging plots on this site had significantly lower cover of low gorse (LG) (Fig. 3a), bare earth/ground (BGRD) (Fig. 3k) *Juncus* rushes

(JU) (Fig. 3m) and open water (OW), than expected from cover at the 50 x 50 m scale.

(iii) Habitat associations with brood size and nestling condition

Brood size was significantly lower at nests with greater mean rock (RK) and heather (HE) (Fig. 3j) cover within 100 m, and significantly higher at nests with presence of tall gorse shrubs (PRESSHRUB) (Fig. 3n) (GLM, n = 96 nests, intercept est = 4.386 ± 0.330 young, HE est = -0.443 ± 0.151, *P* = 0.004, RK est = -1.094 ± 0.392 young, *P* = 0.006, PRESSHRUB est = +0.793 ± 0.303 young, *P*

Table 2. Modelled effects within final generalized linear model (negative binomial error distribution) for predicting log number of foraging visits by meadow pipits to a given 50 x 50 m square (n = 468, 12 per nest) within 100 m of the nest (n = 39). Estimated (log) effect size, standard error, probability values. Nest was also included as fixed factor. PG = pastoral short grass, MG = gorse shrubs 0.75 to 1.5 m height, TU = tufted semi-natural grassland grasses, PRESTREES1 = trees present, BGRD = bare ground. Numbers are factor levels, percentage ranges in brackets are cover in 50 x 50 m squares they represent.

	Estimate (log)	S.E.	*P*
(Intercept)	-4.382	1.659	0.008*
Distance band 2 *(= 50-100 m)*	-2.438	0.229	<0.0001***
PG1 *(present-5 %)*	+5.345	1.154	<0.0001***
MG1 *(present-5 %)*	-0.742	0.366	0.043*
MG2 *(5-25 %)*	-0.068	0.507	0.893
MG3 *(25-100 %)*	-1.741	0.617	0.005**
TU2 *(5-25 %)*	+0.001	0.313	0.997
TU3 *(25-50 %)*	-1.104	0.632	0.081
TU4 *(50-75 %)*	+1.840	0.689	0.008*
PRESTREES1 *(present)*	+0.774	0.269	0.004**
BGRD1 *(present-5%)*	+1.847	0.987	0.061

= 0.010). Nestling condition was not associated with any vegetation variables but was inversely associated with brood size (GLMM, n = 74 nests, intercept estimate -5.010 ± S.E. 0.711 g, TARSUS est. = +0.981 ± 0.029 g, $P < 0.0001$, BROODSIZE est. = -0.460 ± 0.131 g, $P = 0.0009$). Variation of brood size and nestling condition with habitat were also tested specifically at Holne Moor. Brood size increased with mean cover score for bog (BO) and presence of tall gorse shrubs (PRESSHRUB), and negatively correlated with mean cover score of bare rock (RK) and low gorse (LG), within 100 m of the nest (GLM, n=82 nests, intercept estimate = 4.596 ± 0.334 young, BO est = +0.802 ± 0.392, $P = 0.044$, PRESSHRUB est = +1.491 ± 0.362, $P < 0.0001$, RK est = -1.752 ± 0.472, $P = 0.0004$, LG est = -0.700 ± 0.235, $P = 0.004$). For nestling condition, backward selection of variables culminated in a model with significant negative correlation of condition with brood size and positive correlation with mean cover of mixed low gorse and grasses (GG) within 100m of the nest (GLMM, n = 61 nests, intercept estimate -5.968 ± S.E 0.712 g, TARSUS est. = +1.018 ± 0.030 g, $P < 0.0001$, BROODSIZE est = -0.542 ± 0.117 g, $P < 0.0001$, GG est = +0.189 ± 0.063 g, $P = 0.0035$).

Testing how nestling condition varied with foraging plot vegetation, in addition to increasing with tarsus length, nestling mass increased with cover of heather (HE) and large gorse shrub (SHRUB) vegetation and bare rock (RK) on foraging plots, but was inversely associated with cover of medium gorse shrubs (MG), homogeneous tall meadow grass (HO) (Fig. 3f), bare ground (BGRD) and bilberry (BI) (GLMM, n = 23 nests, intercept estimate = -5.600 ± 0.755 g, TARSUS (mm) est. = +0.956 ± 0.036, $P < 0.0001$, SHRUB est. = +8.338 ± 1.828, $P = 0.0068$, RK est. = +1.954 ± 0.664, $P = 0.0185$, HE est. = +0.393 ± 0.148, $P = 0.0250$, MG est. = -0.755 ± 0.280, $P = 0.0229$, HO est. = -0.466 ± 0.122, $P = 0.0022$, BGRD est. = -2.151 ± 0.508, $P = 0.0011$, BI est. = -0.387 ± 0.143, $P = 0.0215$).

(iv) Habitat associations of provisioning behaviour

Provisioning visit rate increased with nestling age (CHICKDAY) and brood size (NCHICK), and was inversely associated with mean cover of homogeneous tall

Table 3. Differences in vegetation cover scores between foraging plots (FPs) and their encompassing (enc.) 50 x 50 m square of habitat, using paired Wilcoxon Signed Rank tests. Sum of ranks V of positive differences in score (score on FP – score on enc.), *P* value, and direction of difference (Diff, "FP < enc." denotes lower score on FPs).

	Holne Moor (n=126 FPs)			Meldon Common (n=32)		
	V	*P*	Diff	V	*P*	Diff
LG	1182	0.0004	FP < enc.	58.5	>0.05	n.s.
MG	219	<0.0001	FP < enc.	49.5	<0.05	FP < enc.
GG	185	<0.0001	FP < enc.	13	<0.05	FP < enc.
GR	5484.5	<0.0001	FP > enc.	258.5	<0.05	FP > enc.
PG	[none found within 100m]			0	>0.05	n.s.
HO	[none found within 100m]			[none found within 100m]		
TU	1155	0.181	n.s.	83.5	>0.05	n.s.
DG	149.5	<0.05	FP < enc.	30.5	<0.05	FP < enc.
BR	716	<0.0001	FP < enc.	12	<0.05	FP < enc.
HE	2336	0.103	n.s.	32	~0.05	n.s.
MO	1319	0.695	n.s.	63	>0.05	n.s.
BI	923	0.401	n.s.	19	<0.05	FP < enc
RK	355	<0.0001	FP < enc.	9	<0.05	FP < enc.
BGRD	682	0.0008	FP < enc.	55	>0.05	n.s.
JU	746	<0.0001	FP < enc.		(not	present)
OW	62.5	<0.05	FP < enc.		(not	present)
BO	119.5	>0.05	n.s.		(not	present)

SHRUB	58	<0.0001	FP < enc.	0	<0.05	FP < enc.
TREE	0	<0.05	FP < enc.	0	<0.05	FP < enc.

meadow grasses (HO) and bracken (BR) on foraging plots. There was a significant negative correlation of rate with time of observation session (START_HR), and session duration (Table 4). Prey load size increased with nestling age (CHICKDAY), and was inversely associated with cloud cover (CLOUD) and cover of dead gorse stalks (DG) and bracken (BR) on foraging plots (Table 4). See appendices for scatterplots relating to these models. Repeating the modelling for Holne Moor alone, the final model for provisioning visit rate was similar with respect to non-habitat variables, but showed an inverse association with bog cover (BO) and no association with HO or BR. The final model for mean prey load size had no significant modelled effect of DG (reported further in chapter 4).

(v) Provisioning rates and nestling stage survival

Daily nest survival of nests that received provisioning observations did not significantly correlate with mean foraging distance, provisioning visit rate, mean prey load size, or the interaction between provisioning visit rate and prey load size (GLMMs, RATE est. (logit) = +0.134 ± 0.316, P = 0.672, n = 38 nests; MEANSIZE est. (logit) = +0.178 ± 1.941, P = 0.927, n = 36 nests; RATE*MEANSIZE est. (logit) = +0.036 ± 0.498, P = 0.943, n = 34 nests; MEANDISTANCE est. (logit) = -0.004 ± 0.027, P = 0.885, n = 40 nests) (Fig. 5). Daily nest survival of these nests also showed no correlation with hatch date (GLMM n = 41 nests, HATCHDATE est. (logit) = -0.013 ± 0.082, P = 0.874).

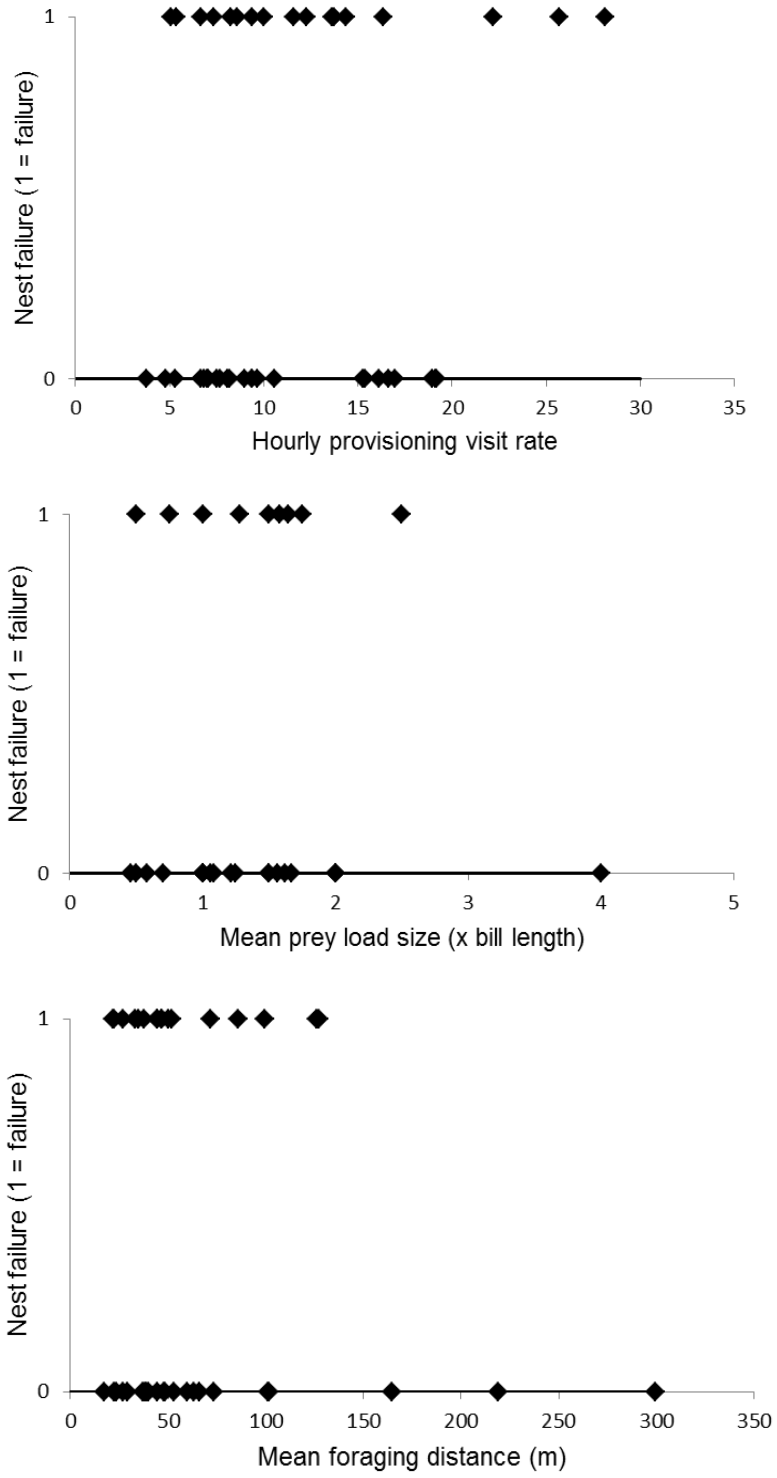


Figure 5. Scatterplots showing nest failure occurrence (1 = failure, 0 = survival to fledging) of nests relative to the a) hourly provisioning visit rate b) mean prey load size and c) mean foraging distance, recorded in their first observation sessions. Line shows modelled relationship of failure with the provisioning variable.

Table 4. Structures of final models predicting variation in meadow pipit nest provisioning behaviour in terms of i) visit rate and ii) mean delivered prey load size, across sites in Dartmoor National Park, Devon UK.

	Model													
Visit rate (/ h)														
	Intercept	+	CHICKDAY	+	NCHICK	+	START_HR	+	DURATION	+	HO	+	BR	
Est.	18.134		+0.501		+2.596		-0.543		-0.214		-1.152		-0.788	
S.E.	5.619		0.164		0.929		0.207		0.047		0.0562		0.366	
P	0.0020		0.0034		0.0080		0.0112		<0.0001		0.0449		0.0357	
Size (relative to bill length)														
	Intercept	+	CHICKDAY	+	$CLOUD_{2,3}$	+	DG	+	BR					
Est.	1.896		+0.087		-0.998 | -0.724		-0.313		-0.159					
S.E.	0.347		0.029		0.230 | 0.225		0.140		0.051					
P	<0.0001		0.0047		<0.0001| 0.0022		0.0298		0.0030					

Discussion

(i) and (ii) Foraging habitat preference

Most foraging trips occurred within 100 m of the nest (Fig. 4), and number of foraging visits made to a square was significantly higher for squares within 50 m of the nest (Table 2). This is consistent with previous studies of provisioning in meadow pipits (Douglas *et al.* 2008, Vandenberghe *et al.* 2009). Central place foragers are expected to minimise distance from the nest if prey can be obtained nearby, both to maximise efficiency of provisioning young and increase ability to defend nests from conspecifics and predators. Meadow pipits selected foraging plots (10 m) with extensive short semi-natural grassland grass, within wider (50 m) areas that contained more complex vegetation, including vegetation types that were statistically avoided on foraging plots. Areas of short sward within semi-natural grasslands have greater visibility and accessibility of invertebrate prey (Butler & Gillings 2004, Brodmann *et al.* 2010). Trees and tall ground vegetation may be avoided on foraging plots because they are more difficult to forage amongst, and they obscure ground-feeding birds' view of surroundings for detecting predators (Whittingham & Evans 2004). However their presence at the 50 m scale may be important for providing source habitat for additional diversity of prey taxa (Arrizabalaga-Escudero *et al.* 2015). The present study indicates preference for heterogeneity at the 50 x 50 m scale in semi-natural grasslands. In the study area, heterogeneity is largely the result of low-intensity grazing pressure. Vandenberghe *et al.* (2009) previously indicated that meadow pipits in upland dominated by purple moor grass *Molinia caerulea* also preferred a mixed grazing regime, and associated heterogeneity in experimental plots of roughly 3.3 hectares. As also noted by Douglas *et al.* (2008), it is not possible to determine whether meadow pipit foraging is influenced primarily by lower predation risk or greater plant and prey biodiversity in areas of vegetation heterogeneity, and achieving this would likely require experimental field studies. The present study also indicates preference for foraging plots with relatively short and uniform sward likely to be caused by patchy grazing. Van Klink *et al.* (2015) found foraging plots of 6 x 6 m in grazed salt marsh (i.e. lowland sites) were more homogeneous in height than random plots at the same distance from the nest. Similarly, meadow pipits in upland

Molinia-dominated grassland selected 2 x 2 m squares of lower vegetation height and density than random squares (Douglas *et al.* 2008 Vandenberghe *et al.* 2009).

The greater frequency of foraging within 50 m of trees and pastoral grassland (Fig. 3e) (Table 2) suggests meadow pipits in the study area are attracted to edge habitats such as the boundary between semi-natural grasslands and enclosed pastore at the hill edge. The results also indicate a preference for, rather than a tolerance of, trees in semi-natural grasslands. Correlation of enclosed pastoral land with meadow pipit foraging is previously reported at similar altitudes by Seel and Walton (1979). However, a positive correlation of foraging with tree presence has not previously been reported perhaps because most meadow pipit study areas involve habitats in which trees are scarce or absent.

(iii) Habitat associations with brood size and nestling condition

Across study areas, brood size was lower at nests with greater mean cover of bare rock and heather, and higher at those with larger gorse shrubs within 100 m. In the study area, increased cover of heather and bare rock co-occurred with acidic soil and dominance of gorse as opposed to grasses. Previous studies have suggested meadow pipit breeding is limited in more homogeneous or ubiquitous heather growth (Vanhinsberg & Chamberlain 2001). While brood size also correlated with gorse-shrub and rock cover at Holne Moor alone, here there were also significant correlations for other vegetation types with brood size, namely low gorse (negative) and (*Sphagnum*) bog cover (positive). Meadow pipit breeding significantly co-occurred with bog cover in previous studies and is suggested to relate to favourable nestling prey availability (Vanhinsberg & Chamberlain 2001). In our study area, bog cover was not significant in predicting use of ground for foraging at the 50 m scale, perhaps due to its relatively rarity across our study areas; and was neither selected nor avoided as a foraging substrate at the 10 m scale (Table 3). Negative correlation of brood size with low gorse is not readily explained. It may represent the breeding strategy or lower fecundity of birds which occupy nest

territories with extensive grass-free gorse patches, but may alternatively be colinearity. Low gorse differs from the main gorse nesting vegetation 'mixed gorse and grass' (GG) in its lack of interspersing growth of grasses such as *Festuca ovina*. The low gorse vegetation category may co-occur with habitat of overall reduced suitability for tall growth of grasses, and with unsuitable conditions (soil, prey abundance) for investment by pipits in large broods. Low gorse and rock were both avoided relative to availability as foraging substrates by pipits (Table 3). Across study areas, nestling condition correlated inversely with brood size and there were no additional or direct vegetation correlates of nestling condition once variation due to brood size was accounted for. This analysis suggests habitat may impact on nestling condition via brood size but not directly through territory-scale effects on prey abundance or foraging efficiency. This is unexpected as the analysis of nestling condition against foraging plot vegetation in analysis (iii) suggested that vegetation cover on foraging plots influenced nestling condition. However this analysis involved a small sample size due to relevant data being available from 2016 only.

In the study area at Holne Moor, nestling condition correlated positively with cover of mixed gorse and grass vegetation surrounding nests. This is the most frequently used nesting vegetation in this study area. Greater extent of potential nesting vegetation may limit ability of nest predators to detect nest sites (Whittingham and Evans 2004). Nest predation risk has been shown to positively correlate with parental activity in other open nesting passerines (Martin *et al.* 2000). Provisioning rates were higher in increased low gorse vegetation (LG) (Table 4) which is of similar structure to the nesting vegetation.

(iv) Habitat associations of provisioning behaviour

Modelling provisioning visit rate across both sites, there was a significant positive correlation with nestling age and brood size, but also negative correlation with cover of long meadow grass (HO) and bracken (BR) on foraging plots (Table 4). Provisioning rate is expected to be higher for larger broods due to greater absolute energy requirements, and to increase as nestlings develop due to increasing daily energy requirements. Homogeneous tall meadow grass

HO areas were consistently further than 100 m from all observed nests, therefore birds using this habitat were undertaking longer foraging distances which may explain significantly lower provisioning visit rates where this foraging habitat was used. In analysis (ii), bracken was statistically avoided by foraging meadow pipits relative to wider availability (Table 3) but commonly co-occurred with patches of short grasses GR which were selected by meadow pipits (Table 3). Bracken grows as stiff shoots and when mature creates shade patches, both of which may reduce efficiency of prey searching on foraging plots. Duration and start time of observation sessions also had a significant negative effect on provisioning rate (Table 4). Time of observation is expected to correlate with rate as a result of changing activity level through the day. Activity of other insectivorous birds corresponded with availability (not necessarily flight activity) of their insect prey (Hutto 1981). However, duration most likely correlated simply as a result of the observation methodology, because birds undertaking trips at a greater rate, to a greater number of different foraging locations would result in earlier termination of observation sessions.

Mean prey load size increased with nestling age, and was negatively associated with cloud cover and more extensive dead gorse twig (Fig. 3h) and bracken on foraging plots (Table 4). Prey load size is expected to increase with nestling development, as nestlings have greater daily energy requirements for growth and become capable of ingesting larger individual prey as they grow. Cloud cover limits direct sunlight reaching the ground and is likely to reduce visual detection of all invertebrates, as well as limiting temperature on the ground and therefore activity of many invertebrate taxa. While cloud and rain measurements in observation sessions did not strongly correlate (Pearson's r = 0.33), higher cloud cover was more likely to occur and persist during periods with intermittent rain outside of observation sessions that would wet vegetation and the ground. Cloud cover may therefore reflect moisture level which has a significant influence on invertebrate activity and bird feeding behaviour (Elkins 2010). Cloud cover did not significantly correlate with provisioning visit rate, which suggests cloud cover did not reduce foraging time, for example by increasing time at the nest brooding nestlings. Bracken was previously modelled to significantly reduce nest provisioning rates. In the study areas, dead or burnt

gorse stalks and bracken on foraging plots do not block grazing from occurring, and therefore are commonly associated with undergrowth of short semi-natural grassland grasses. This substrate was statistically selected by pipits relative to availability in the present study (Table 3) and is widely reported as preferred for pipit foraging (Douglas *et al.* 2008, Vandenberghe *et al.* 2009). Dead gorse stalks and bracken were overall statistically avoided by foraging meadow pipits relative to availability (Table 3) but their co-occurrence with short grasses makes them likely to occur on foraging plots. The presence of dead gorse or bracken causes a taller effective sward height, which is shown in experimental studies to increase foraging birds' time allocation to vigilance for predators (Whittingham & Evans 2004). Greater occurrence of dead gorse or bracken on foraging plots may reduce prey loads by limiting proportion of time spent searching, reducing efficiency and number of prey caught per foraging trip.

Provisioning was observed at a distance therefore size of individual prey items could not be accurately recorded, but reduced prey load size may have been influenced by smaller prey items being collected. Prey item size could be lower in greater cloud cover due to weather effects on activity or visibility of large prey. For example adult moths, that constituted some of the largest individual prey recorded during observations, were more visible or active in times with direct sunlight (pers. obs.).

(v) Provisioning rates and nestling stage survival

Daily failure probability of observed pipit nests did not significantly correlate with provisioning visit rate, mean prey load size, interaction between these two variables or mean foraging distance (Fig. 5). Provisioning rates may have not shown a significant relationship with nest survival as most nest failures, where cause could be established, were caused by predation. Predation was therefore considered relatively important at the study site compared to failure from insufficient food provisioning. Critically, this analysis could only include nests that had survived to receive at least one (nestling) provisioning observation session. As a result, nests failing at the early nestling stage would often be excluded and variation in nest failure rate would be expected to be reduced

relative to the whole sample of nests with nestlings. The study did not include nesting density in analyses, and investigate density dependence of effects. Breeding bird surveys carried out across the study seasons suggested abundance of adults per km^2 showed little annual variation. Peak adult counts in transects of two 1 km squares at Holne Moor were 58 (2015), 53 (2016) and 48 (2017). Measurement of nest density would be valuable to future studies of a similar nature, to properly account for potential density-dependence.

Land use and meadow pipit foraging

Gorse at its dwarf-shrub stage of succession is used for nesting by meadow pipits and is restrictive to movement and foraging of grazing animals. Meadow pipit nests in gorse may be at lower risk of destruction from trampling by herbivores than in grassland without gorse. By restricting grazer access to all areas of grass in unenclosed uplands, mature gorse is partly responsible for ensuring heterogeneity of grassland vegetation structure, which at 50 m scale was indicated to be favoured by meadow pipits.

Short grass was the favoured substrate for foraging in the present study, and this has been previously recorded for meadow pipit (Douglas *et al.* 2008, Vandenberghe *et al.* 2009), as well as skylark *Alauda arvensis* and starling *Sturnus vulgaris* (Vickery *et al.* 2001). Grazing by sheep or cattle provides the preferred physical structure of vegetation for meadow pipit foraging in this study, as in previous studies (Douglas *et al.* 2008, Vandenberghe *et al.* 2009). Grazing on the study site is seasonal and of sufficiently low intensity to permit heterogeneous grassland sward height. In lowland agricultural grasslands more accessible to use of machinery, artificial cutting of grasslands, for example for silage production, uniformly reduces the sward to a height of seemingly high suitability for meadow pipit foraging, but leaves no sward of sufficient height for nesting. Silage cutting usually first takes place in May in the UK (Vickery *et al.* 2001), which is the peak month for meadow pipit nesting (Rose 1982). Compared to cutting of hay, which due to requirements of dry weather normally succeeds the end of pipit nesting, the increase in silage relative to hay production has not been conducive to safe meadow pipit breeding in cut

grasslands. While intensified grazing can similarly reduce entire areas to a uniformly short sward, grazing involves dunging and involves selective grazing of plant taxa, both of which may help to provide relatively beneficial foraging conditions for meadow pipits. While dung from grazers is patchy in application, increased application of fertilizers that are often applied homogeneously to the whole land parcel can reduce grasslands' suitability as a foraging substrate for meadow pipits, through shifting grassland vegetation assemblage to fast-growing, uniform and dense in character.

Overall the present study supports previous findings that meadow pipits prefer heterogeneous habitat structure at the 'field' scale (50 m) and short homogeneous patches of grasses at the foraging site scale (10 m), which are likely to exist under low intensity grazing. Bracken and dead (or burnt) gorse stalks were selected against by foraging meadow pipits, and were negatively associated with prey load sizes, but both vegetation types also frequently occurred on patches of short grass vegetation selected by meadow pipits. Dead gorse stalks are primarily the product of managed burning, and bracken is subject to management by physical 'bashing' in the late summer but grows without intervention at many sites during spring and summer. Our analyses suggest increased availability of short grass areas free of bracken growth or intact gorse post-burning would increase availability of primary foraging habitat for meadow pipits and could facilitate capture of greater prey load sizes per foraging visit.

References

Arrizabalaga-Escudero, A., Garin, I., García-Mudarra, J. L., Alberdi, A., Aihartza, J., and Goiti, U. (2015) Trophic requirements beyond foraging habitats: The importance of prey source habitats in bat conservation. *Biological Conservation* 191: 512–519.

Atkinson, P. W., Fuller, R. J., Vickery, J. A., Conway, G. J., Tallowin, J. R. B., Smith, R. E. N., Haysom, K. A., Ings, T. C., Asteraki, E. J., and Brown, V. K. (2005) Influence of agricultural management, sward structure and food

resources on grassland field use by birds in lowland England. *Journal of Applied Ecology* 42(5): 932–942.

Balmer, D., Gillings, S., Caffrey, B., Swann, B., Downie, I., and Fuller, F. (2013) *Bird Atlas 2007–11.* Thetford: BTO.

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67(1): 1–48.

Bibby, C. J., and Thomas, D. K. (1985) Breeding and diets of the Reed Warbler at a rich and a poor site. *Bird Study* 32(1): 19–31.

Bishton, G. (2001) Social structure, habitat use and breeding biology of hedgerow Dunnocks *Prunella modularis*. *Bird Study* 48(2): 188–193.

Boersma, P. D., and Rebstock, G. A. (2009) Foraging distance affects reproductive success in Magellanic penguins. *Marine Ecology Progress Series*. Inter-Research Science Center.

Braun-Blanquet, J. (1932) *Plant sociology. The study of plant communities.* New York: McGraw-Hill.

Brodmann, P. A., Reyer, H.-U., and Baer, B. (2010) The Relative Importance of Habitat Structure and of Prey Characteristics for the Foraging Success of Water Pipits (*Anthus spinoletta*)*. *Ethology* 103(3): 222–235.

Brooke, M. D. L., and Davies, N. B. (1987) Recent changes in host usage by cuckoos Cuculus canorus in Britain. *Journal of Animal Ecology* 56(3): 873–883.

BTO (1994) *BTO/JNCC/RSPB Breeding Bird Survey instructions*. British Trust for Ornithology.

Buchanan, G. M., Grant, M. C., Sanderson, R. a., and Pearce-Higgins, J. W. (2006) The contribution of invertebrate taxa to moorland bird diets and the potential implications of land-use management. *Ibis* 148(4): 615–628.

Butler, S. J., and Gillings, S. (2004) Quantifying the effects of habitat structure on prey detectability and accessibility to farmland birds. *Ibis* 146: 123–130.

Chance, E. P. (1922). *The cuckoo's secret*. Sidgwick and Jackson, Limited.

Cramp, S. (1988) Handbook of the birds of Europe, the Middle East and North Africa: the birds of the western Palearctic. Oxford, UK: Oxford University Press.

Crawley, M. J. (2012). *The R book.* John Wiley and Sons.

Davies, J., Arthur, D., and White, S. (2014) Effects of variation in breeding habitat on Ring Ouzel Turdus torquatus productivity and chick condition. *Bird Study* 61(2): 162–170.

Douglas, D. J. T., Bellamy, P. E., Stephen, L. S., Pearce–Higgins, J. W., Wilson, J. D., and Grant, M. C. (2014) Upland land use predicts population decline in a globally near-threatened wader. *Journal of Applied Ecology* 51(1): 194–203.

Douglas, D. J. T., Evans, D. M., and Redpath, S. M. (2008) Selection of foraging habitat and nestling diet by Meadow Pipits *Anthus pratensis* breeding on intensively grazed moorland. *Bird Study* 55(3): 290–296.

Elkins, N. (2010) *Weather and bird behaviour*. (Third edit.). London: Bloomsbury Publishing.

Evans, D. M., Redpath, S. M., and Evans, S. A. (2005) Seasonal Patterns in the Productivity of Meadow Pipits in the Uplands of Scotland. *J. Field Ornithol.* 76(3): 245–251.

Evans, D. M., Redpath, S. M., Evans, S. A., Elston, D. A., Gardner, C. J., Dennis, P., and Pakeman, R. J. (2006) Low intensity, mixed livestock grazing improves the breeding abundance of a common insectivorous passerine. *Biology Letters* 2(4): 636–638.

Gibbons, D.W., Reid, J.B. and Chapman, R.A. (1993) *The New Atlas of Breeding Birds in Britain and Ireland: 1988 –1991.* T. and A.D. Poyser, London, UK.

Glue, D., and Morgan, R. (1972) Cuckoo Hosts in British Habitats. *Bird Study* 19(4): 187–192.

Grim, T., and Honza, M. (2001) Does supernormal stimulus influence parental behaviour of the cuckoo's host? *Behavioral Ecology and Sociobiology* 49(4): 322–329.

Hågvar, S., Glesne, O., and Østbye, E. (2009) Food habits and niche overlap in three alpine passerine birds, South Norway. *Ornis Norvegica* 32: 56–73.

Harris, S. J., Massimino, D., Gillings, S., Eaton, M. A., Noble, D. G., Balmer, D. E., Procter, D., and Pearce-Higgins, J.W. and Woodcock, P. (2018) *The Breeding Bird Survey 2017. BTO Research Report 706*. Thetford.

Hewson, C. M., Thorup, K., Pearce-Higgins, J. W., and Atkinson, P. W. (2016) Population decline is linked to migration route in the Common Cuckoo. *Nature Communications* 7: 12296.

Hutto, R. L. (1981). Temporal patterns of foraging activity in some wood warblers in relation to the availability of insect prey. *Behavioral Ecology and Sociobiology*, 9(3): 195-198.

IUCN 2017. *The IUCN Red List of Threatened Species. Version 2017-3*. <http://www.iucnredlist.org>. Downloaded on 05 December 2017.

Jacobsen, L. B., Jensen, N. O., Willemoes, M., Hansen, L., Desholm, M., Fox, A. D., Tøttrup, A. P., and Thorup, K. (2017) Annual spatiotemporal migration schedules in three larger insectivorous birds: European nightjar, common swift and common cuckoo. *Animal Biotelemetry* 5(1): 4.

Kleintjes, P. K., and Dahlsten, D. L. (1992) A Comparison of Three Techniques for Analyzing the Arthropod Diet of Plain Titmouse and Chestnut-Backed Chickadee Nestlings. *Journal of Field Ornithology* 63(3): 276–285.

Kleven, O., Moksnes, A., Røskaft, E., and Honza, M. (1999) Host species affects the growth rate of cuckoo (*Cuculus canorus*) chicks. *Behavioral Ecology and Sociobiology* 47(1–2): 41–46.

Kosicki, J. Z., and Chylarecki, P. (2013) Predictive mapping of Meadow Pipit density using integrated remote sensing data and an atlas of vascular plants dataset. *Bird Study* 60(4): 500–508.

Kuznetsova, A., Brockhoff, P., and Christensen, R. (2017) lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* 82(13): 1–26.

Martin, T.E., Scott, J., and Menge, C. (2000) Nest predation increases with parental activity: separating nest site and parental activity effects. *Proceedings of the Royal Society of London B: Biological Sciences* 267(1459): 2287–2293.

Massimino, D., Woodward, I.D., Hammond, M.J., Harris, S.J., Leech, D.I., Noble, D.G., Walker, R.H., Barimore, C., Dadam, D., Eglington, S.M., Marchant, J.H., Sullivan, M.J.P., Baillie, S.R. and Robinson, R. A. (2017) *BirdTrends 2017: trends in numbers, breeding success and survival for UK breeding birds. Research Report 704.* Thetford.

Moreby, S. J., and Stoate, C. (2000) A quantitative comparison of neck-collar and faecal analysis to determine passerine nestling diet. *Bird Study* 47(3): 320–331.

Pearce-Higgins, J. W., and Grant, M. C. (2006) Relationships between bird abundance and the composition and structure of moorland vegetation. *Bird Study* 53(2): 112–125.

Pearce-Higgins, J. W., Grant, M. C., Beale, C. M., Buchanan, G. M., and Sim, I. M. W. (2009) International importance and drivers of change of upland bird populations. In Bonn, A., Allott, T., Hubacek, K., and Stewart, J. (Eds.), *Drivers of Environmental Change in Uplands.* London/New York: Routledge.

Razeng, E., and Watson, D. M. (2015). Nutritional composition of the preferred prey of insectivorous birds: popularity reflects quality. *Journal of Avian Biology*, 46(1): 89-96.

Rose, L. N. (1982) Breeding ecology of British pipits and their Cuckoo parasite. *Bird Study* 29(1): 27–40.

Šálek, M., Chrenková, M., Dobrý, M., Kipson, M., Grill, S., and Václav, R. (2016) Scale-dependent habitat associations of a rapidly declining farmland predator, the Little Owl *Athene noctua*, in contrasting agricultural landscapes. *Agriculture, Ecosystems & Environment* 224: 56–66.

Sharrock, J. T. R. (1976). *The Atlas of Breeding Birds in Britain and Ireland.* Poyser, Calton.

Smith, A. A., Redpath, S. M., Campbell, S. T., and Thirgood, S. j (2001) Meadow pipits , red grouse and the habitat characteristics of managed grouse moors. *Journal of Applied Ecology* 38: 390–400.

Soler, M. (2008) Do hosts of interspecific brood parasites feed parasitic chicks with lower-quality prey? *Animal Behaviour* 76(5): 1761–1763.

Thorup, K., Tøttrup, A. P., Willemoes, M., Klaassen, R. H. G., Strandberg, R., Vega, M. L., Dasari, H. P., Araújo, M. B., Wikelski, M., and Rahbek, C. (2017) Resource tracking within and across continents in long-distance bird migrants. *Science Advances* 3(1): 1–11.

van Klink, R., Mandema, F. S., Bakker, J. P., and Tinbergen, J. M. (2014) Foraging site choice and diet selection of Meadow Pipits *Anthus pratensis* breeding on grazed salt marshes. *Bird Study* 61(1): 101–110.

Vandenberghe, C., Prior, G., Littlewood, N. a., Brooker, R., and Pakeman, R. (2009) Influence of livestock grazing on meadow pipit foraging behaviour in upland grassland. *Basic and Applied Ecology* 10(7): 662–670.

Vanhinsberg, D. P., and Chamberlain, D. E. (2001) Habitat associations of breeding Meadow Pipits *Anthus pratensis* in the British uplands. *Bird Study* 48(2): 159–172.

Vickery, J. A., Tallowin, J. R., Feber, R. E., Asteraki, E. J., Atkinson, P. W., Fuller, R. J., and Brown, V. K. (2001) The management of lowland neutral grasslands in Britain: effects of agricultural practices on birds and their food resources. *Journal of Applied Ecology* 38(3): 647–664.

Vickery, J. A. and Arlettaz, R. (2012) The importance of habitat heterogeneity at multiple scales for birds in European agricultural landscapes. In Fuller, R. J. (Ed.), *Birds and Habitat: Relationships in Changing Landscapes.* Cambridge: Cambridge University Press.

Vickery, J.A. and Fuller, R.J. (1998) Use of Cereal Fields by Birds: A Review of Relation to Field Margin Management. BTO Research Report No. 195. British Trust for Ornithology, Thetford, UK

Walton, K. C. (1979) Diet of meadow pipits *Anthus pratensis* on mountain grassland in Snowdonia. *Ibis* 121(3): 325–329.

Whittingham, M. J. and Evans, K. L. (2004). The effects of habitat structure on predation risk of birds in agricultural landscapes. *Ibis* 146: 210-220.

Wilson, J. D., Arroyo, B. E., and Clark, S. C. (1996) *The diet of bird species of lowland farmland: a literature review. Unpublished report to the Department of the Environment and Joint Nature Conservation Committee*. Sandy, Bedfordshire.

Wyllie, I. (1981) *The Cuckoo*. London: Batsford.

4. Provisioning of nestlings and parasitic common cuckoo *Cuculus canorus* nestlings in grassland by meadow pipits *Anthus pratensis*

Abstract

Nesting success of insectivorous birds depends on invertebrate food supply in addition to nesting habitat. Parasitic common cuckoo *Cuculus canorus* nestlings hatch from eggs laid into the nests of invertebrate-feeding passerine 'hosts'. Cuckoos are declining in many parts of their Palaearctic breeding range. To understand and halt these declines, identification of key food and habitat resources to cuckoo nestlings is critical, requiring studies comparing their ecology of that of host nestlings. Another key question is whether cuckoo nestlings receive greater provisioning input than host broods (indicating greater energetic demand). Previous research suggests cuckoos are provisioned at a similar rate to host broods, with some difference in prey composition. However, existing research covers a small subset of host species, and hosts raising cuckoos may shift their foraging distances or habitat use to maintain visit rates. In Britain where cuckoos are declining, the meadow pipit *Anthus pratensis* is the main host in uplands, a relative stronghold habitat. The present study aimed to determine if nestling cuckoos in uplands receive different or additional resources from hosts. The meadow pipit nesting and foraging habitat, provisioning activity (visit rate, prey load size, foraging distance, habitat selection, provisioned prey), and daily nest survival associated with cuckoo-parasitised nests were compared with those associated with non-parasitised nests. Nests with more extensive bracken and water and less extensive *Juncus* and tufted long grass cover within 100 m had higher occurrence of cuckoo parasitism. Host pairs raising a cuckoo nestling showed similar selection and exclusion of foraging vegetation, and showed no significant difference in provisioning visit rate, prey load size or foraging distance, to pairs raising their own young. Cuckoo fledglings were provisioned at a higher rate (with no decrease in prey load size) and with greater foraging distances than during their nestling stage, and than broods of meadow pipit nestlings. Raising a cuckoo is

indicated to involve a greater absolute expenditure of energy from host meadow pipits (and at later stages a greater rate of expenditure) than raising a brood of conspecific young. Cuckoo nestlings and fledglings were overall provisioned a greater diversity of invertebrates than meadow pipit broods, with greater frequency of Diptera of all sizes, and of dipteran families Chloropidae and Rhagionidae.The study suggests that resource requirements of the cuckoo in grasslands are different to those of meadow pipit broods. Greater diversity of available prey was indicated to be important for hosts to provision a cuckoo. Lower within-sample species richness for cuckoo faeces suggests host pairs variously select specific alternative prey for a super-sized cuckoo nestling, rather than foraging less discriminately due to larger cuckoo growth requirements. However this observation should be treated with caution due to the difficulty of determining diet diversity of individuals from faeces. Management measures which promote invertebrate biomass as well as biodiversity may be effective in conserving breeding populations of cuckoo and meadow pipit.

Introduction

Resource availability during breeding is a key driver of nest-stage demography, which can critically drive change in animal populations (e.g. Lewis *et al.* 2001). Identifying key resources (food, habitat) used during breeding is therefore a vital area of conservation science. The common cuckoo *Cuculus canorus* has undergone significant decline in adult numbers, especially in western parts of its Palaearctic breeding range (PECBMS 2019). It is an obligate brood parasite, with nestlings raised from egg to independence by host songbird pairs at their nest. The cuckoo is also an Afro-Palaearctic migrant, spending the Northern Hemisphere winter in sub-Saharan Africa. The “multiple jeopardy” from relying on resources across many geographic stages in their annual cycle makes identification of key drivers of population decline difficult, as causal factors at one stage may take effect on a demographic rate at a subsequent stage, referred to as a carry-over effect (Newton 2004). For example, processes in the non-breeding period have been suggested to influence cuckoo populations via

adult survival, as regional cuckoo population change in Britain correlates with proportions of local tracked birds that use a 'western' southbound migration route through Iberia rather than an 'eastern' route through Italy and the Balkans (Hewson *et al.* 2016). Tracked birds using the western route show greater mortality, concentrated in the Iberian Peninsula. Variation in other non-breeding factors between breeding populations is limited, with tracked adults from across the Palaearctic breeding range shown to migrate to similar areas of southern Africa for the Northern Hemisphere winter, and cuckoos from declining and stable populations across western Europe taking similar northbound migration routes (Willemoes *et al.* 2014, Hewson *et al.* 2016, Birding Beijing 2018, Thorup *et al.* 2018). Juveniles have also been tracked to the same regions as adults for their first overwintering period (Vega *et al.* 2016). However, birds that migrate south via the western route also depart later (Hewson *et al.* 2016), and within Britain as well as the wider breeding range, cuckoos breed in a range of habitats targeting a large diversity of passerine hosts (Glue & Morgan 1972, Moksnes & Røskaft 1995). Variation in southbound migration route is therefore made up of multiple confounding factors and the breeding ground stage is potentially one of the greatest sources of variation among populations within the annual migration cycle. Meanwhile cuckoos show differing long-term trends in breeding population between European countries (PECBMS 2019) and UK regions (Morrison *et al.* 2013, Harris *et al.* 2018) and in the UK have strongly declined and become dissociated from agricultural habitats whilst remaining stable in semi-natural habitats (Massimino *et al.* 2017, Denerley *et al.* 2018). Understanding of the full annual cycle of the common cuckoo is required to increase ability to identify causes of population trends. While tracking has improved knowledge of non-breeding stages, monitoring of populations and body condition of birds at different staging areas will need to be improved further. Variation in trend between breeding populations despite limited spatial variation in non-breeding regions, suggest a role of breeding ground factors in driving populations.

Study of cuckoo demography and resource use in the Palaearctic breeding season is also of high value as it is when reproduction occurs and thus may be critical to conservation effort. However, this is particularly challenging due to the

brood parasitic life style. Ecology of the nestling cuckoo is closely associated with that of the host, and linked to that of the adult cuckoo primarily by the habitat and nest site in which the egg is initially laid. Cuckoo nestling survival is likely to be vulnerable to all threats faced by the host songbirds during their nesting. Locating cuckoo-parasitised nests often requires extensive detection of many host nests. Tracking suggests the cuckoo breeding season is exceptionally short among large landbirds, due to lack of parental care, with northern European individuals measured to remain an average of 49 days in the area where they breed (Willemoes *et al.* 2014).

Across its Palaearctic breeding range, the cuckoo parasitises a large diversity of songbird host species (Moksnes & Røskaft 1995) which vary in feeding habitat and nestling diet. In the UK there are three common host species (Glue & Morgan 1972) – meadow pipit, dunnock *Prunella modularis* and reed warbler *Acrocephalus scirpaceus*. Meadow pipits and dunnocks forage principally on the ground (Seel & Walton 1979, Bishton 1986) while reed warblers forage above ground or water amongst woody or reed vegetation (Catchpole 1972, Bibby & Thomas 1985). Meadow pipits provision a diversity of invertebrates to nestlings (Walton 1979, Wilson *et al.* 1996), largely favouring the most abundant but selecting prey relative to accessibility and size (Douglas *et al.* 2008, van Klink *et al.* 2014). Reed warblers are considered similarly to be generalist (Bibby & Thomas 1985). Published consensus of major prey orders fed to nestlings of host species are: Meadow pipit: Lepidoptera larvae plus Diptera (commonly larvae of family Tipulidae) (Walton 1979, Evans *et al.* 2005, Douglas *et al.* 2008, Hågvar *et al.* 2009); Dunnock: Coleoptera, Diptera and more variably Lepidoptera and Araneae (Bishton 1985, Moreby & Stoate 2001); Reed warbler: Diptera, Araneae, Coleoptera and Hemiptera (Bibby & Thomas 1985, Brooke & Davies 1989). This implies significant variation in cuckoo nestling diet between hosts and therefore across the cuckoo's global breeding range.

Within a single host species, cuckoo nestlings may be expected to be provisioned the same prey, at the same rate, as host broods as they mimic host young (Wyllie 1981). However, the cuckoo nestling solicits host provisioning with a begging call rate that first matches and later exceeds that of a whole

brood of host young (Davies *et al.* 1998, Kilner *et al.* 1999). Additionally, nestling body mass later in development exceeds that of a combined host brood (Wyllie 1981, Grim & Honza 2001). Foster pairs may respond to this unique stimulus with shifts in foraging behaviour. A louder begging call in particular was hypothesised to stimulate greater provisioning rate from hosts (Brooke & Davies 1989), limited mainly by increased predation risk of loud begging. While in one study cuckoo nestlings were provisioned more prey mass per unit time than single host nestlings of the same mass (Grim & Honza 2001), this involved comparing provisioning rate to cuckoo and host nestlings at different days of development, and focused on an experimental host brood size of one. In studies comparing provisioning of cuckoos to that of average-size host broods, provisioning visit rate (nest visits per unit time) did not differ (Brooke & Davies 1989, Kilner *et al.* 1999). Prey loads in the bills of reed warblers did not differ when provisioning cuckoos compared to when provisioning their own young (Brooke & Davies 1989). Volume of food brought per hour did not significantly differ between parasitised and unparasitised nests of rufous-tailed scrub robin *Cercotrichas galactotes* (Martín-Gálvez *et al.* 2005), and increased linearly with body mass in both cuckoo and reed warbler nestlings (Grim & Honza 2001). Overall, the studies suggest that a nestling cuckoo can elicit the same prey provisioning rate as an average-size broods of host young, often of four nestlings. However, no study has compared parental foraging distance between cuckoo nestlings and host broods, which may be an area where cuckoos' foster parents compensate to maintain similar feeding rates to those under unparasitised conditions. The most frequently studied cuckoo hosts are *Acrocephalus* warbler species of reedbed and wetland which are less readily observed at distance while foraging around the nest. Where hosts of open habitat have been studied (Chance 1940, Nakamura & Miyazawa 1997), this has not included observation of hosts' foraging movements.

Additionally, prey provisioned to cuckoos and hosts may be of different taxa (Brooke & Davies 1989) and therefore different nutritional value (Soler 2008). Composition of prey is most directly represented using dominance of each prey taxon. Dominance is the percentage of all individuals of all prey taxa consumed that is accounted for by a given prey taxon. However, this requires accurate

counting of prey individuals. Frequency is an alternative measurement of prey occurrence in diets, and is the proportion of all samples (such as pellet or faecal samples), sampling events (such as nest visits), or sample locations (such as nests) where a taxon occurred. This can be calculated across a greater range of methodologies as it relies only on detection of taxa as opposed to counts of individuals of each taxon. Mean dominance per nest of invertebrates fed to cuckoos and rufous-tailed scrub robin nestlings (Martín-Gálvez *et al.* 2005) suggested individual cuckoo young are fed a greater proportion of "larvae" (of unidentified insect orders), plant items (grapes), adult Coleoptera, adult "Homoptera" and arachnids, and a lower proportion of Orthoptera than host nestlings. These results illustrate that under most study methodologies, different taxa vary in the ability and degree to which they can be identified. A cuckoo nestling reared in a mixed brood with great tit *Parus major* young received prey with greater dominance of Lepidoptera larvae and lower dominance of arachnids than great tit nestlings (Grim *et al.* 2014) but this is a singular cuckoo host interaction with a rarely recorded host. In Grim and Honza's (2001) study of cuckoos and reed warbler nestlings, dominance of aphids (Sternorrhyncha), and Heteroptera and Gastropoda were higher in cuckoo nestling diet. Diptera, arachnids, Coleoptera and Hymenoptera were marginally more dominant in reed warbler nestling diet. It is notable from the above that dominances are calculated by proportion of individuals and so may inflate within the study sample data, and diverge between prey compositions of parasite and host nestlings, more rapidly with taxa that are small and delivered in large numbers per provisioning visit, as is likely the case with Sternorrhyncha. In contrast to Martín-Gálvez *et al.* (2005), Grim and Honza (2001) also reported frequency of prey types. Frequencies of all prey taxa were higher among cuckoo prey sampling events (samples of food collected from the gapes of nestlings wearing temporary neck ligatures) than among those from reed warblers, indicating mean number of different taxa was higher per cuckoo sample, and that individual cuckoos were fed a greater diversity than individual reed warbler nestlings (Grim & Honza 2001). Brooke and Davies (1989) previously compared frequency of invertebrates in faeces of cuckoos and reed warblers by microscopy. Coleoptera, Lepidoptera, Heteroptera, Odonata and Araneae had greatest relative frequency among cuckoo faeces compared to host faeces,

while Gastropoda, “Homoptera” and small Diptera were lowest frequency among cuckoo faeces relative to host faeces. The approach of analysing digested remains in faeces rather than intact prey in bill contents meant numbers of prey individuals could not be consistently quantified between faeces and between taxa, so dominances were not calculated. This additionally highlights that varying methods of study complicate comparisons of prey between studies.

Overall, in contrast to rates of provisioning visits and volume of prey per hour, past research suggests that prey provisioned to cuckoo nestlings as opposed to host nestlings differ in proportion and prominence of invertebrate taxa, which could reflect significant differences in parental effort or investment, and vulnerability to environmental impacts on invertebrate groups. Prey composition has differed between studies, and these studies have covered only some of the most frequent host species cited by Wyllie (1981) although frequency of some of these species as cuckoo hosts may have since declined. Prey showing some consistency in being provisioned to cuckoos relatively more than host broods are Araneae, Lepidoptera, Hemiptera and Coleoptera. These are diverse widespread taxa which may simply be frequent prey due to presence in many microhabitats. They may be provisioned more to a nestling with larger nutritional demands (such as a large cuckoo) due to being abundant and accessible on ground and foliage. Studies have typically not classified prey to finer detail than order, which is more likely to present problems when subsequent studies show subsets of this taxon to be potentially important, or the classification is subsequently considered obsolete, as with Homoptera above. Identification to finer taxonomic levels (genus, species) is a means of better protecting study data from this, but is generally not possible for invertebrate prey identified from video recordings, digested remains in faeces or microscopy of intact prey en masse as were the cases here. ‘Molecular biology’ methods, that detect prey through matching DNA sequences from remains in faeces or pellets to known sequences, have evolved through ‘DNA barcoding’ and more recently ‘next-generation sequencing’ approaches, to make identification of prey to genus or species level frequently possible in diet ecology studies (Pompanon *et al.* 2012).

Further to the question of whether nestling diet differs in parasite nestlings from hosts' own broods, is whether provisioned diet significantly affects parasite nestling fledging success and survival. Cuckoo nestlings were shown to have higher growth rate and fledging mass when raised by the larger host species present (great reed warbler *Acrocephalus arundinaceus*). This was true whether cuckoos hatched in the nest of this host, or were translocated from nests of the smaller host (reed warbler), suggesting host species was the primary influence on growth (rather than higher quality adult cuckoos parasitising nests of great reed warblers) and that this could subsequently influence post-fledging survival (Kleven *et al.* 1999). Great reed warblers may generally provision larger prey to young but the study did not measure this. Subsequent studies have reported fledging ages and masses of cuckoos (Grim & Samaš 2016). Several studies have quantified fledging success of cuckoos as percentage of single cuckoo eggs found by observers that resulted in fledging (Kleven *et al.* 2004, Antonov *et al.* 2007, Sklepowicz & Halupka 2009, Trnka *et al.* 2012, Thomson *et al.* 2016), typically on the assumption that eggs are independent when in fact several eggs (including eggs never found) across nests in an area are likely to have been laid by the same female. The influence of female quality is therefore ignored. Only one such study compared daily nest survival rates between parasitised and unparasitised nests, with nests containing cuckoos showing lower survival (Sklepowicz & Halupka 2009). None of the above studies have included detail of nestling diet or provisioning rate for cuckoo or host young. The post-fledging period is amongst the least studied of cuckoo life stages (Tyller *et al.* 2018) and no study has compared provisioning rate, prey taxa or fledging weight with daily survival of cuckoo fledgings. Such studies typically require daily tracking of individuals (Cox *et al.* 2014).

In the previous chapter, meadow pipit brood size, foraging and provisioning in upland semi-natural grasslands were shown to significantly relate to vegetation cover at the 10 m or 50 m spatial scale. Here the nest habitat, foraging habitat selection, provisioning, and nestling stage survival rates were compared between parasitised and unparasitised nests of meadow pipit, to indicate whether raising a cuckoo requires greater host resource (provisioning) or distinct habitat resources (habitat and prey types) than raising a host brood.

Specifically, the following questions were addressed: (i) Does occurrence of parasitism at meadow pipit nests vary with surrounding vegetation cover? (ii) Does foraging habitat preference differ for pipits raising a cuckoo nestling compared to those raising their own young? (iii) Does provisioning visit rate, prey load size or foraging distance differ between cuckoo-parasitised pipit nests and unparasitised nests? (iv) Does provisioning visit rate, prey load size or foraging distance differ between nestling and post-fledging stages of raising a cuckoo? (v) Does the prey composition provisioned by meadow pipits differ between pipit broods and cuckoo nestlings? (vi) Does nestling stage daily nest survival differ between parasitised and unparasitised nests? I applied a similar methodology for foraging observations to previous studies of meadow pipits, to build on existing methods for comparing provisioning of cuckoo and host nestlings and include study of foraging trips during provisioning for the first time. For determining nestling diet I applied molecular (DNA) analysis techniques to nestling faecal samples. This is the first application of this method to the common cuckoo.

Methods

Study area

Meadow pipit nests were located at sites in Dartmoor National Park, UK, in April to July of 2015-2017. Nests were found by extensively searching the site on foot and watching adult meadow pipits with food fly back to incubating females or young at the nest. Some females were watched flying back to eggs. A small minority of nests were found by chance flushing of the female while passing a nest, or by watching a female cuckoo dropping to a nest site to find or parasitise a nest. Most nests were therefore found at the nestling stage. The primary site where work was conducted was Holne Moor (50° 31’ 20” N, 03° 51’ 43” W, altitude 300-400m). The site comprises semi-natural grassland (*Festuca ovina*, *Agrostis capillaris*, *A. curtisii*) with gorse (*Ulex sp.*) facing ancient oak *Quercus sp.* and birch *Betula sp.* woodland to the north and pastoral farmland to the east, and it receives mixed grazing from sheep, cattle and ponies. At least two female cuckoos parasitised nests on the site in a given year, determined from

ringing of individuals and from colour and pattern of located eggs, and parasitism rate was approximately 6% of meadow pipit nests found. The secondary site, Meldon Common (50° 39’ 40” N, 03° 50’ 52” W) is a smaller semi-natural grassland area separate from the main moor, of 300-400 m altitude, bordered on all sides by pastoral grassland agriculture. Unparasitised nests were located and faecal samples collected from broods at two other semi-natural sites; Warren House (50° 36’ 43” N, 03° 52’ 13” W) and Burrator (50° 31’ 24” N, 04° 0’ 55” W).

Provisioning observations were carried out at Holne Moor in 2016 and 2017, and at Meldon Common in 2016. Analysis comparing provisioning at parasitised and unparasitised nests used data only from Holne Moor. Faecal samples were collected from nests at Holne Moor in 2015-17, at Meldon Common in 2015-16 and at Burrator and Warren House in 2015 only.

Nest monitoring and faecal sampling

Visits to monitor nest contents were carried out every 2-4 days unless weather was cold and wet, from the date the nest was found until outcome (success or failure) was established. Age of nestlings (day post-hatching) was based on hatch date when this was known or estimated from state of feather development. Feather growth stage (referred to as CHICKSTAGE in statistical analysis) was recorded following British Trust for Ornthology (BTO) Nest Record Scheme methodology as NA (naked, no feather pin), IP (feather pin, no emergence of barbs in primary feathers), FS (primary feather barbs emerged and grown up to 1/3 of full length) and FM+ (primary feathers more than 1/3 of full length). Pipit nestlings were handled for up to two minutes between one and three times between ages 3 and 10 days; cuckoo nestlings between ages 3 and 15 days, under Natural England license (two handlings) or BTO Ringing Scheme license (one handling for ringing). Faecal samples released incidentally during handling were collected directly into 5 ml vials, punctured with a sterile Pasteur pipette, and covered with 80-90% ethanol. Faecal samples from a whole pipit brood were pooled per visit. Samples were refrigerated at 5° C within 24 hours (but see Discussion).

Nest and fledgling observations

I carried out all observations of provisioning behaviour at nests during their nestling stage, at a distance allowing both a view of the wider foraging area and a telescope view of the size of food carried by adult birds to the nest. Time of visit by a parent bird, plus prey load size as a proportion of bill length, and location of foraged plots of habitat were recorded. Foraging plot locations were recorded with handheld GPS immediately after observation sessions. Observations were carried out at the same hour of day for a given nest, ranging from 0700 to 1900 BST. Observation sessions ran for 60 min or until five distinct foraging plots were seen to be used; whichever was soonest. Nests located at earlier stages were prioritised for faecal sampling and observation, to increase opportunity for repeat sampling and observation. For each observation session the mean rate of feeding visits per hour was calculated as rate = 60 / t, where t is the mean time period in minutes between observed feed visits during the session. This was used rather than counting feeding visits per hour of observation, as birds were predicted to be disturbed from normal provisioning rate for a variable period at the beginning of observation sessions. This was assumed in addition to only commencing observations when no alarm calling was heard or disturbance was visible.

Past studies suggested a shift in foster parent provisioning behaviour at latter stages of the cuckoo's development (Grim & Honza 2001), therefore fledgling cuckoos were also observed while still dependent on the host pair, to maximise the available period in which to quantify provisioning of large cuckoo young. The exact perching location of a fledgling was determined on as many days as possible, by listening for begging calls in an increasing radius around the nest and by watching adults carrying food. In practice this was possible up to approximately 400 m from the nest and up to 22 days after fledging. Once located, fledglings were observed identically to the method used for nests. The location of the observer was GPS recorded. Locations of up to five observed adult foraging plots and the fledgling itself were noted at a distance during observations. These locations were GPS recorded after the observation session and after the fledgling had vacated the perch, respectively, to avoid disturbance. Cloud cover, rain, wind and visibility during the observation session were

recorded by scores of 1 to 3 following BTO Breeding Bird Survey methodology (BTO 1994).

Vegetation measurement

Vegetation in a 100 m radius around each nest, and in 10 x 10 m squares centred on each observed foraging location, was measured between 1 and 31 August to avoid disturbance to breeding birds. 10 m squares at foraging locations are subsequently referred to as foraging plots. The 100 m radius was identified as suitable from previous literature (Douglas *et al.* 2008, Vandenberghe *et al.* 2009). Vegetation variables relating to major vegetation and substrate types were split where appropriate into height classes to capture habitat structure (chapter 3, Table 1). Vegetation coverage was measured in 12 squares of 50 x 50 m in a cross formation (chapter 3, Fig. 2). Vegetation cover was recorded the same in 50 x 50m squares and 10 x 10m foraging plots, using an adapted Braun-Blanquet scale of 0 to 5 representing percentage cover (0 = 0%/absent; 1 = up to 5 %, 2 = 5-25 %; 3 = 25-50%; 4 = 50-75%, 5 = 75=100%) (Braun-Blanquet 1932). Percentage cover estimates treated each habitat variable as a layer of up to 100% cover, with its value unaffected by presence of overlying layers (e.g. bracken growing over grasses). Presence or absence of gorse shrubs over 1.5 m high, and trees over 1.5 m and over 3 m high, were also recorded.

Prey DNA sequencing

Prey of meadow pipit and cuckoo nestlings were identified from DNA sequencing of faecal samples collected at nests under Natural England license or under BTO Ringing Scheme license during ringing. To compare prey of cuckoo and meadow pipit nestlings, a ‘next-generation’ amplicon sequencing method (as in Bohmann *et al.* 2011) was applied using an Illumina MISEQ system. Samples included were cuckoo nestling and fledgling faeces and a targeted subset of meadow pipit brood faecal samples collected on matching or similar dates, selecting for each cuckoo sample the meadow pipit sample collected at closest proximity within the previous or subsequent two days. In subsequent text this analysis is referred to as the ‘comparative’ prey analysis. The meadow pipit samples selected here were part of a significantly larger

sample of brood faeces collected across the four sites on Dartmoor. To obtain a large baseline data set on meadow pipit prey composition, prey were sequenced from the remaining 81 pipit nestling faecal samples (combined per brood) from 63 nests (18 nests were sampled twice) across all four study sites, using a clone and (Sanger) sequence approach (as in King *et al.* 2015). This is hereafter referred to as the 'baseline' prey analysis. Both segments of prey analysis focused on amplifying, and determining the sequences present in each faecal sample, of a 157-212 base pair (bp) length region of the cytochrome-b oxidase I (COI) gene found in animal mitochondrial DNA (mtDNA). Sequencing of DNA extracted from organisms of known identity shows this region to be significantly conserved within, and variable between, animal taxa, commonly at the level of species (Brown 1985). The gene region is therefore established to provide a 'barcode' DNA sequence (Hebert *et al.* 2003).

For the baseline prey analysis, DNA was extracted from faecal samples using QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). DNA of the target COI region was amplified through PCR (polymerase chain reaction) using primers previously designed and used for generalist invertebrate COI amplification (LCO1490 and HCO1777, see Folmer *et al.* 1994 and Brown *et al.* 2012; ZBJ-ArtF1c and ZBJ-ArtR2c, see Zeale *et al.* 2011). Purified PCR products were ligated to pGEM-T Easy Vector (Promega, Madison, USA) which were taken up by competent *Escherichia coli* cells which were plate cultured. The cloned invertebrate DNA sequence of at least 10 colonies per faecal sample was amplified with Sp6 and T7 long primers, and Sanger sequenced against T7 primer.

For the comparative prey analysis, DNA was extracted using a precipitation and re-suspension method (Chaput unpublished 2017) as this was found in tests following the baseline study to have higher DNA yield. Libraries for amplicon sequencing were prepared by amplifying invertebrate DNA from each extract via PCR using the generalist primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al.* 2011). In each PCR, primers were modified to carry a unique 5' adaptor sequence, to allow reference between sequences and original samples following MISEQ runs. DNA sequence 'reads' from Illumina MISEQ runs were de-multiplexed according to the 5' adaptor used in PCR of DNA from each

faecal sample. In the R package Dada2 (Callahan *et al.* 2016), sequences were clipped of the first 30 bp and 24 bp from the forward and reverse reads of sequences, respectively, to remove primer sequences. Reads were truncated at 200 bp and truncated where base call accuracy fell below Q35. Reads were dereplicated in Dada2 which was used to identify amplicon sequence variants (ASVs). Each ASV was entered as a query to a search of all species-level barcodes (3,181,157 sequences, 192,424 species, as of July 2018) in the Barcode of Life Database (BOLD) version 4 (Ratnasingham & Hebert 2007). Species barcode sequences were considered matches if similarity exceeded 97% (following Stackebrandt & Goebel 1994). The species with the highest similarity above this threshold was recorded following King *et al.* (2015). Species identifications for each unique sequence, and the total number of reads in each faecal sample were exported into a summarising table. All ASV sequences from MISEQ with similarity of database sequences greater than 97% (following Stackebrandt and Goebel 1994) were retained. For detailed methodology of molecular analyses see chapter 2.

Data analysis

i) Comparing vegetation associations of parasitised and non parasitised nests

In order to test whether occurrence of nest parasitsm was associated with vegetation surrounding nests, a generalized linear model with binomial error distribution and logit link was fitted to test whether vegetation variables significantly predicted the response of whether a nest was unparasitised (0) or parasitised by cuckoo (1). Cover scores of each vegetation variable (and presence scores of presence variables) were pooled to a mean score across the 12 50 m squares surrounding the nest, so that each nest had one mean value of each vegetation variable. Nest parasitism (1 or 0) was the response variable and vegetation variables were included as covariates. Model selection proceeded by backward selection from the 'full-model'. The correlation matrix of all potentially included variables was first inspected, and all variable pairs or groups covarying with Pearson's correlation coefficient $r \geq 0.7$ magnitude (±) were highlighted for potential exclusion. Each variable in a pair or group was then tested alone against the response of parasitism, and the variable with

lowest probability value *P* was retained and all others excluded from model selection. Backward selection then proceeded from the full model, consulting the type III (ANOVA) output for each successive model composition and deleting the variable of highest probability value, until all variables included in the model were significant at $P < 0.05$.

In order to test the concordance of this approach with an alternative data reduction approach, this analysis was repeated using a principal components analysis. Variation in mean cover of each habitat variable per 12 squares within 100 m around each nest was summarised in a total of 18 principal components. The variation for which principal components accounted was examined. The components explaining the first and second most variation were scatter plotted and the resultant clustering of nests containing cuckoo versus meadow pipit nestlings was examined. The magnitude of correlation of each habitat variable with the first principal component (PC1) was examined and ranked from largest to smallest.

(ii) Comparing foraging habitat selection of pipits raising cuckoo or own brood

Most identified 10 x 10 m foraging plots were predicted to lie within 100 m of the nest based on previous provisioning observation of meadow pipits (Douglas *et al.* 2008, Vandenberghe *et al.* 2009). In chapter 3 the habitat cover scores in 10 x 10 m squares and their encompassing 50 x 50 m squares were used to test whether habitat variables were significantly selected or excluded from foraging plots by non-parasitised meadow pipit pairs. Here, in order to test the foraging habitat selection of parasitised pairs at the Holne Moor site, identical testing was carried out using vegetation cover scores from each identified 10 m square and their encompassing 50 m square within 100 m of parasitised nests. For each vegetation variable, a paired Wilcoxon Signed Rank test (with continuity correction) was run to compare the scores at the two scales. Each test reported the summed rank *V* of positive differences in cover score for a vegetation variable. A higher value of *V* for a vegetation type signified greater percentage cover on 10 m foraging plots than in surrounding 50 m squares, lower values signified lower cover on foraging plots. These respectively indicated selection or avoidance of vegetation relative to availability. The array of selected and

excluded habitats among i) non-parasitised pairs at Holne Moor (from chapter 3) and ii) parasitised pairs at Holne Moor was then inspected to identify variables where results differed in statistical significance and indicated orientation of preference.

(iii) Comparing provisioning of cuckoo nestlings and host broods

In order to test the relationship of provisioning behaviour with foraging habitat and whether a pair was raising a cuckoo or a host brood, I repeated the modelling and backward selection process as described in chapter 3 analysis iv using data for non-parasitised nests at Holne Moor only, to determine the best generalized linear mixed model structure for predicting response variables of (1) hourly provisioning visit rate and (2) mean prey load size per observation session. These models were run with data at the level of observation session with nest identity as a random factor, and foraging plot vegetation cover scores across all visits to all identified foraging plots per observation session pooled to a single mean value per observation session. Mean foraging vegetation variables were included as covariates. Additionally for this study, I carried out modelling with the response variable of (3) foraging plot distance from the nest, with data at the level of individual nest provisioning visit, and both nest identity and observation session identity as random factors. Foraging plot vegetation variables were categorical fixed factors (as they were not pooled to mean values) and scores of low frequency (≤10 occurrences) were pooled with the adjacent score so that each score accounted for more than 10 surveyed 10 m squares. All of the above models proceeded through backward selection as described in analysis (i) of this study.

For each measure of provisioning behaviour (1)-(3), the above best model structure was then run with the full sample of both parasitised and unparasitised nests at Holne Moor, with parasitism status (SPECIES) (meadow pipit nestlings = 0 or parasitic cuckoo nestling = 1) added as a fixed categorical factor. Before proceeding to this stage, the variables of the full sample dataset were checked for collinearity and variables excluded using Pearson's correlation coefficients and univariate tests as above. In adding SPECIES as a categorical factor to assess difference in provisioning for parasitised nests, meadow pipit broods

were compared to ‘broods’ of one (cuckoo) which have a longer nestling period than pipit broods. The variables for number of young NCHICK and nestling age (day post-hatching) CHICKDAY were therefore assumed to covary with SPECIES. On adding SPECIES, NCHICK was therefore excluded if already present in the model, and CHICKDAY was replaced with the categorical feather growth factor CHICKSTAGE which allowed comparable measurement of nestling development between cuckoo and meadow pipit. Having applied the best model for unparasitised nests to the full sample and added SPECIES, backward selection proceeded as above. Finally, forward selection of the model structure was conducted to detect and add further explanatory variables to the model. Each potential additional variable was fitted in turn, referring to the type III outputs throughout and selecting the variable with lowest probability value in each round for inclusion. This was repeated until no additional variables could be added with significance at the $P < 0.05$ critical value. In practice this added one further variable of significance to the models for mean prey load size and foraging distance.

(iv) Comparing provisioning of pipit and cuckoo nestlings and cuckoo fledglings

To test whether (1) provisioning visit rate, (2) prey load size or (3) provisioning distance of adult meadow pipits differed during the fledgling stage of raising a cuckoo, the correlation matrix of variables for data from all observation sessions included in analysis (iii) and additionally all observation sessions of cuckoo fledglings, was consulted. Collinearity was resolved as above. To this dataset the full model previously used as a starting point for backward selection in analysis (iii) was fitted, replacing SPECIES with a three level fixed factor YOUNG (meadow pipit brood, cuckoo nestling, cuckoo fledgling), for predicting provisioning visit rate, mean prey load size and foraging plot distance. If CHICKSTAGE was initially present in the full model this was removed as feather stage was expected to covary between nestling and fledgling stages of the cuckoo (all fledglings had similarly advanced feather stage). For each of these response variables the model proceeded through backward selection followed by forward selection as in analysis (iii).

(v) Comparing prey fed to nestling meadow pipits and cuckoo young

For the baseline analysis, of meadow pipit diet from a larger sample of broods, the total set of sequences including replications read from cloning of faecal DNA, were entered in the web-based CD-HIT-EST (Li & Godzik 2006, Ying *et al.* 2010) to identify the unique COI-region sequences across all faecal samples from which cloning took place. These sequences and their reverse complements were entered in batches as queries to a BOLD version 4 search, specifying a search of species-level barcode records. I assigned a best matching OTU (operational taxonomic unit) to each sequence based on the unifying taxonomic classification of the ten species-level barcode matches with over 98% similarity (following Clare *et al.* 2011 and King *et al.* 2015) (or all barcodes matching over 98% if fewer than 10). If all database matches originated from taxa with no known UK occurrence, the first taxonomic level upwards containing members of known UK occurrence was used. OTUs were linked back to all matching sequences and onward to faecal samples, using CD-HIT-EST cluster results.

Presence of taxa in faecal samples was used to determine presence of taxa at each nest. This was done across faecal samples used in both the baseline and comparative studies. Frequency was calculated across samples and across nests, for samples from cuckoos and meadow pipit broods in the comparative prey study, and meadow pipit broods in the baseline analysis.

Using samples sequenced in the comparative (MISEQ) analysis, in order to test whether each arthropod order or family detected by the study was more prevalent in nests with a cuckoo nestling or meadow pipit brood, I used a generalized linear mixed model with binomial error distribution to test the linear relationship between nestling species (cuckoo or meadow pipit) and the logit probability of occurrence of invertebrate orders and families which had been detected in four or more faecal samples. For each order or family a model was fitted at the replication level of sample MISEQ run, with occurrence (1 = detected) as the response. Species (meadow pipit = 0, cuckoo = 1) was included as a fixed factor, and sample identity nested within nest identity as a random factor. Using the combined MISEQ reads of each taxonomic family per

sample, Shannon diversity index was calculated per cuckoo and meadow pipit faecal sample. Accumulation curves based on 100 permutations were generated for the set of cuckoo and meadow pipit faecal samples in the comparative analysis. Using the combined MISEQ reads of each taxonomic family per species (meadow pipit or cuckoo), a rarefaction approach was taken to estimate family diversity per 100 000 reads (used here in place of individuals) for faecal samples from each species.

(vi) Nest survival rates of parasitised and unparasitised nests

To test whether daily failure during the nestling stage varied between parasitised and unparasitised nests, I used a generalized linear mixed model where the outcome variable FAIL denoted whether the nest failed (1) or successfully fledged (0), as determined from nest monitoring. I calculated the number of days of the nestling period for which the nest was monitored. The start date was when the nest was found, if found at the nestling stage, or the estimated hatch date if the nest had been found prior to eggs hatching. The end point was when the nest was estimated to have failed or fledged, which if not known with certainty was the half way point (to the nearest 0.5 days) between the last visit with live nestlings and the first visit with no live nestlings. The model contained FAIL as a response variable (fail = 1), with log number of days monitored as an offset, and nestling species (SPECIES) as a categorical fixed factor. Analyses of effect of provisioning rate on daily survival, carried out in the previous chapter, ultimately tested survival of nests that had already survived to receive provisioning observations. To test how survival in this subgroup of nests differed with parasitism, I repeated the above modelling with the smaller sample of 47 nests that received observation sessions to follow the analysis in chapter 3.

All statistical analyses were conducted using R version 3.5.0 software. Mixed modelling was carried out using the R package *lme4* (Bates *et al.* 2015) with probability values estimated using *lmerTest* (Kuznetsova *et al.* 2017). Shannon diversity, accumulation curves and rarefaction were carried out using the R package *vegan* (Oksanen *et al.* 2019).

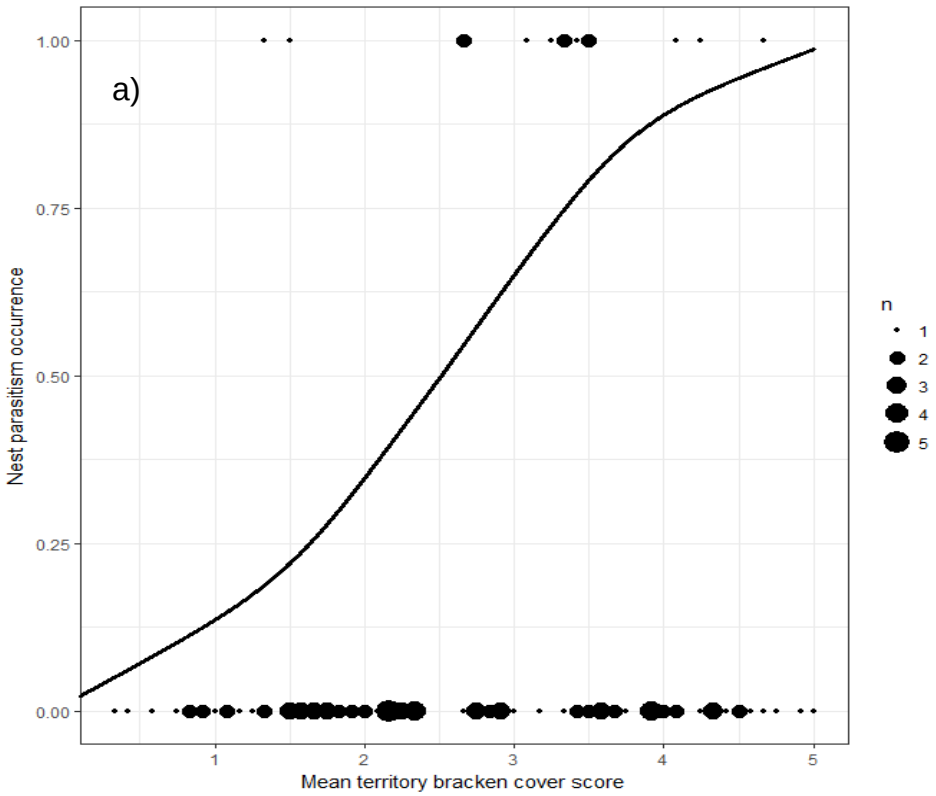
Results

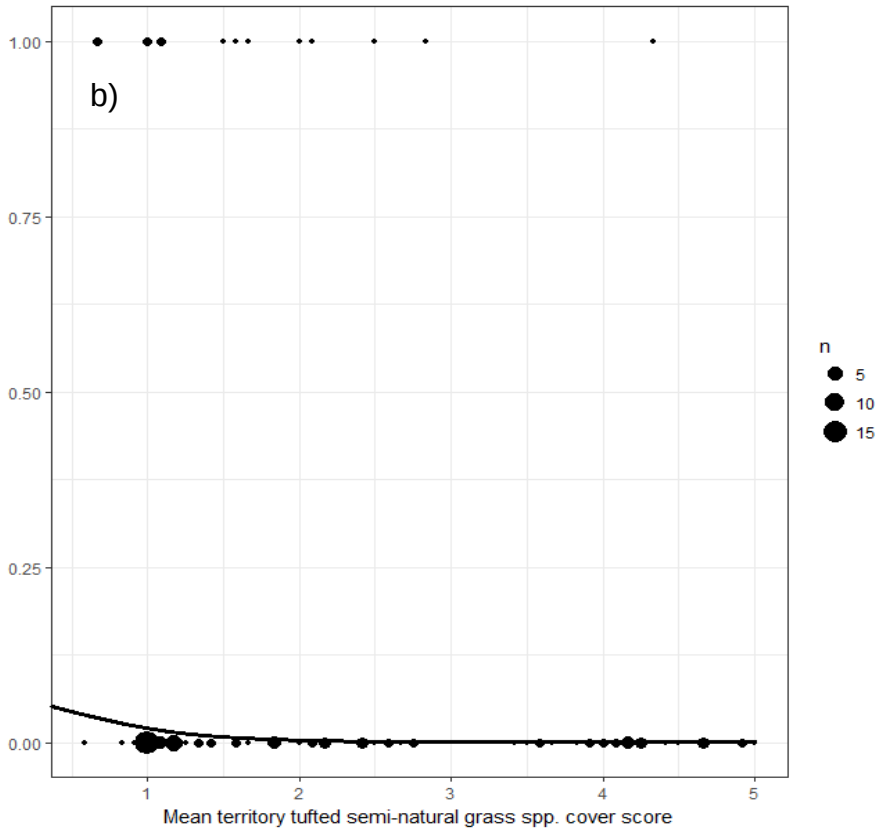
At Holne Moor, habitat was recorded in a 100 m radius around 89 unparasitised meadow pipit nests, and 14 nests parasitised by cuckoo, found over the three seasons 2015-2017. Over the 2016 and 2017 seasons, 73 nest observation sessions (57 of 30 unparasitised nests, 16 of 6 parasitised nests) were carried out at Holne Moor, totalling 63.9 h (51 h of unparasitised nests, 12.9 h of parasitised nests). An additional 9 observation sessions totalling 6.85 h were carried out on 3 fledgling cuckoos from 3 of the parasitised nests. 81 whole or partial brood faecal samples from meadow pipits at 63 nests (56 Holne Moor, 3 Burrator, 2 Warren House and 2 Meldon Common) were collected for the baseline prey analysis. 11 faecal samples from nestling or early fledgling cuckoos from 5 nests at Holne Moor, and 12 meadow pipit brood faecal samples from 12 nests from Holne Moor and Meldon Common, were collected for the comparative prey analysis.

i) Comparing vegetation associations of parasitised and non parasitised nests

Nests with higher mean cover score of bracken (BR) and open water (OW) within 100 m radius, and lower mean cover of tufted semi-natural grassland grasses (TU) and *Juncus* rushes (JU), were significantly more likely to be parasitised by common cuckoo (generalized linear model, n = 103 nests, intercept probability estimate (logit) = -2.473 ± 1.22, BR est = +1.137 ± 0.39, P = 0.004, OW est. = +5.344 ± 2.20, P = 0.015, TU est. = -1.074 ± 0.43, P = 0.012, JU est. = -2.675 ± 1.30, P = 0.040) (Fig. 1). There was low colinearity among these habitat variables (Appendix 6).

In principal component analysis, the first principal component (PC1) accounted for 25.0% of variation variation in mean habitat cover within 100 m of nests, and PC2 explained 18.3% (Appendix 7). The habitat variables of greatest absolute correlation with PC1 were mixed low gorse and grass (GG, +0.3981), moss (MO, -0.3589) and bilberry (BI, -0.3312). The variables of greatest correlation with PC2 were presence of tall gorse shrubs (PRESSHRUB, -0.4284), bare rock (RK, -0.3727), tufted semi-natural grassland grasses (TU, 0.3531) and low gorse (LG, -0.3356). A subset of nests parasitised by cuckoo formed a cluster in the scatter


a)
Nest parasitism occurrence
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
Mean territory bracken cover score
n
1
2
3
4
5


b)
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
Mean territory tufted semi-natural grass spp. cover score
n
5
10
15

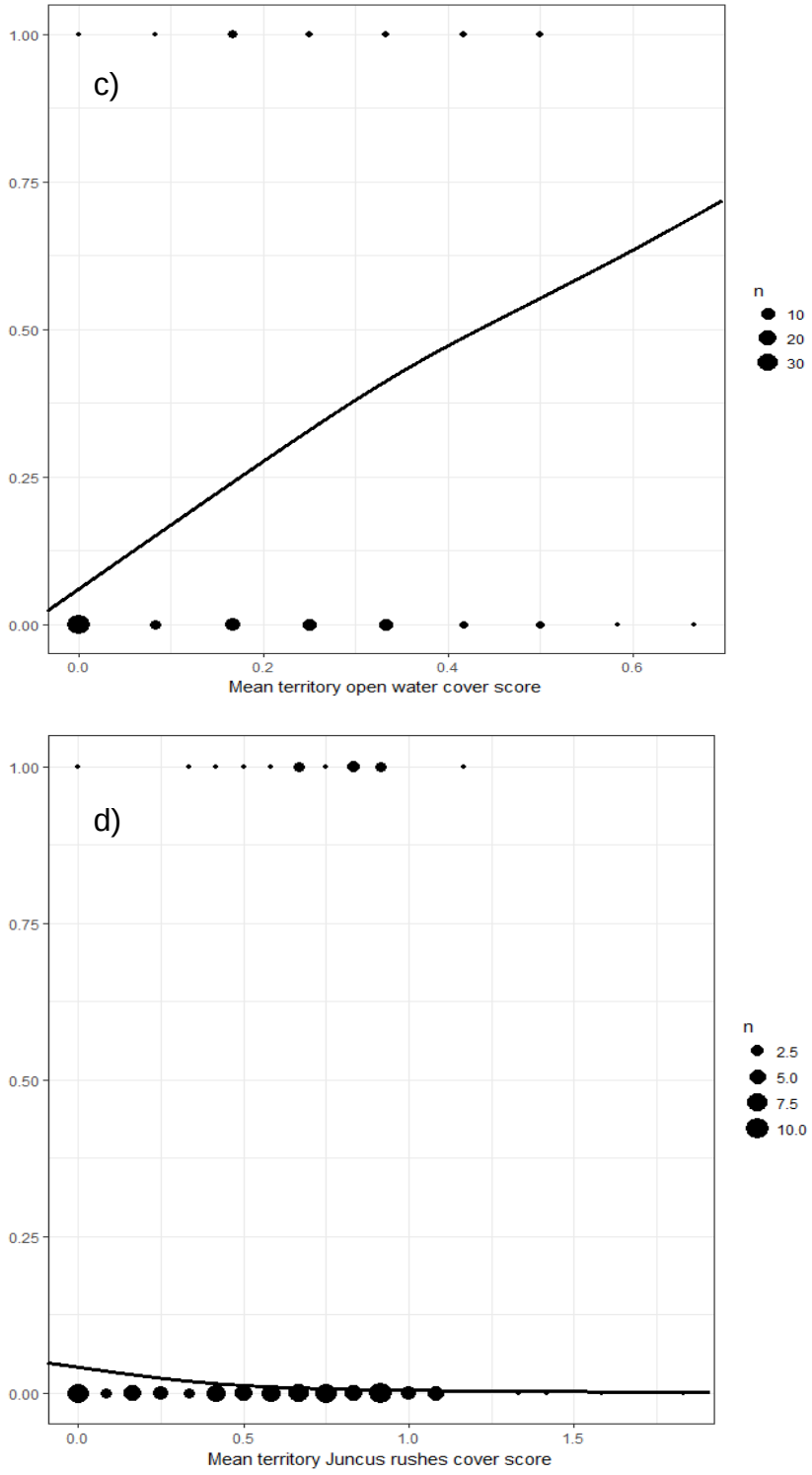


Figure 1. Scatterplots showing occurrence of cuckoo parasitism relative to mean cover of a) bracken *Pteridium aquilinum*, b) tufted semi-natural grassland grasses, c) open water and d) *Juncus* rushes, within 100 m of nests. Lines are modelled variation in probability of parasitism with each vegetation variable when cover of the three other vegetation types is constant.

plot of PC1 vs PC2, but parasitised and unparasitised nests did not generally show strong segregation with respect to these principal components (Appendix 8).

(ii) Comparing foraging habitat selection of pipits raising cuckoo or own brood

Meadow pipits provisioning a cuckoo nestling made 90% of foraging plot visits within 100 m of the nest (n = 91, quartile 1 = 41 m, median = 50 m, q_3 = 76 m). This compared to 78.8% of foraging plot visits for meadow pipits provisioning their own brood (n = 302, q_1 = 20 m, median = 45 m, q_3 = 77 m.)

Within 100 m of the nest, pipits provisioning meadow pipit broods and pipits provisioning cuckoo nestlings both foraged in 10 m squares with significantly greater cover of short semi-natural grassland grasses (GR), and with significantly lower cover of gorse vegetation (LG, MG, GG, SHRUB), bracken (BR), bare rock (RK), *Juncus* rushes (JU), open water (OW), and trees than predicted from cover in surrounding 50 m squares (Table 1). Exclusion of dead gorse (DG) and bare ground (BGRD) from foraging plots relative to availability in 50 m squares was statistically significant for pipit pairs raising their own young but not pairs raising a cuckoo nestling (Table 1).

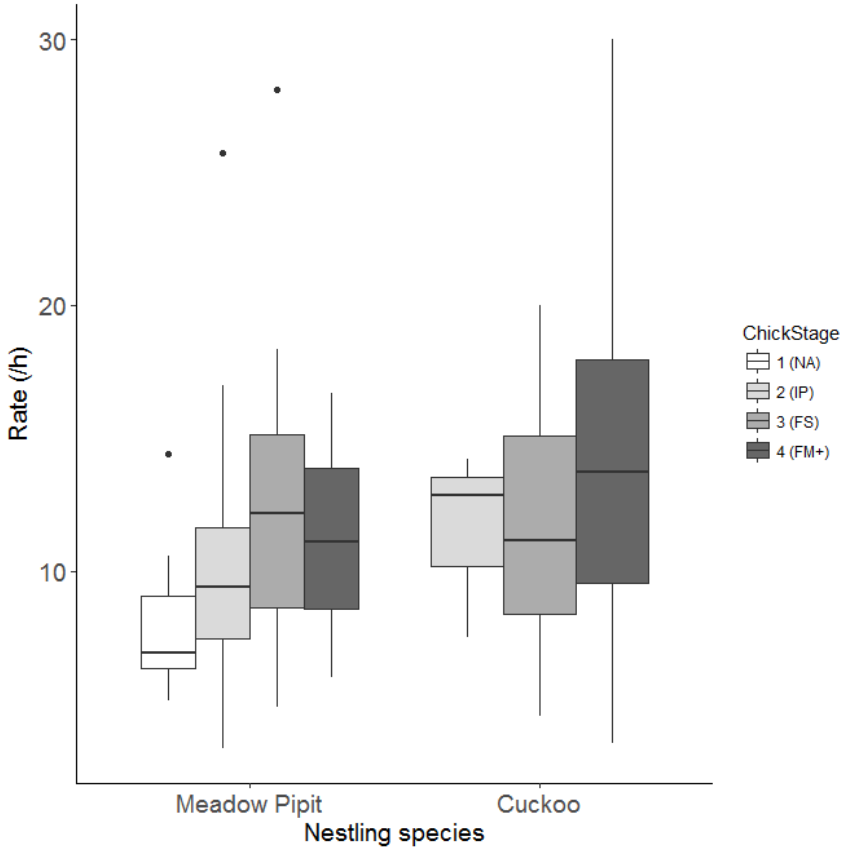
(iii) Comparing provisioning of cuckoo nestlings and host broods

Candidate models for best describing variation in nest provisioning behaviours at unparasitised meadow pipit nests are summarised in Table 2. These models were selected using data from 57 observation sessions across 31 nests. Nest provisioning rate showed significant increase with nestling age (CHICKDAY) and brood size (NCHICK). Additionally rate was negatively associated with later

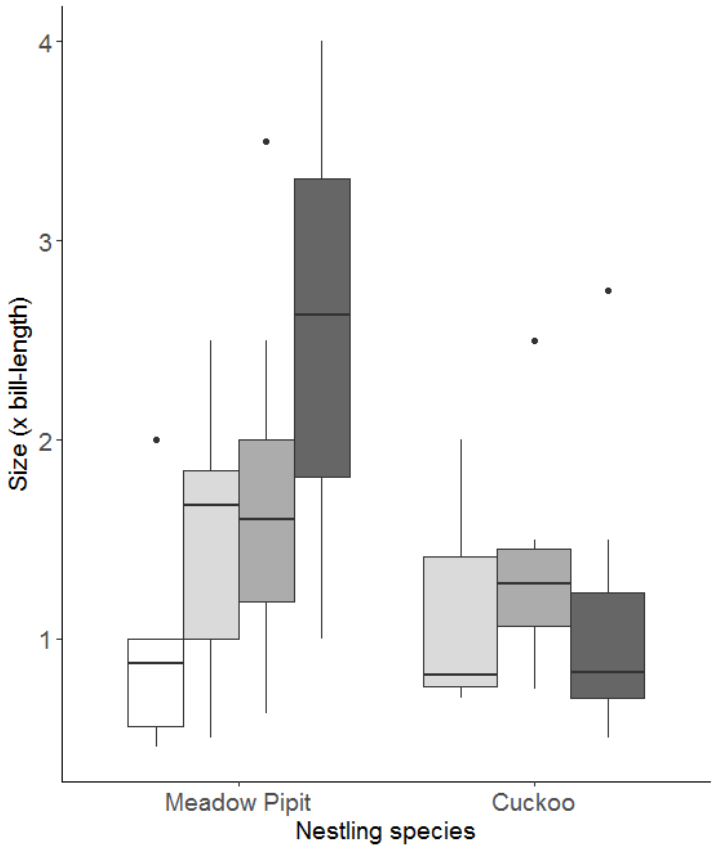
Table 1. Differences in vegetation cover between foraging plots (FPs) and their encompassing (enc.) 50 m square of habitat, using paired Wilcoxon Signed Rank tests, for unparasitised and cuckoo-parasitised meadow pipit nests. Sum of ranks *V* of positive differences in score (score on FP – score on enc.), *P* value, and direction of difference (Diff) (FP < enc. denotes lower cover on FPs).

	Unparasitised (n=126 FPs)			Parasitised (n=35 FPs)		
	V	*P*	Diff	V	*P*	Diff
LG	1182	0.0004	FP < enc.	31	<0.05	FP < enc.
MG	219	<0.0001	FP < enc.	23	<0.05	FP < enc.
GG	185	<0.0001	FP < enc.	75	<0.05	FP < enc.
GR	5484.5	<0.0001	FP > enc.	188	<0.05	FP > enc.
TU	1155	0.181	non-sig.	147.5	>0.05	n.s.
DG	149.5	<0.05	FP < enc.	26	>0.05	n.s.
BR	716	<0.0001	FP < enc.	67.5	<0.05	FP < enc.
HE	2336	0.103	n.s.	136	>0.05	n.s.
MO	1319	0.695	n.s.	114	>0.05	n.s.
BI	923	0.401	n.s.	22	>0.05	n.s.
RK	355	<0.0001	FP < enc.	0	<0.05	FP < enc.
BGRD	682	0.0008	FP < enc.	40	>0.05	n.s.
JU	746	<0.0001	FP < enc.	36.5	<0.05	FP < enc.
OW	62.5	<0.05	FP < enc.	5	<0.05	FP < enc.
BO	119.5	>0.05	n.s.	2	>0.05	n.s.
SHRUB	58	<0.0001	FP < enc.	0	<0.05	FP < enc.
TREE	0	<0.05	FP < enc.	0	<0.05	FP < enc.

a)


30
20
10
Rate (/h)
Meadow Pipit
Cuckoo
Nestling species
ChickStage
1 (NA)
2 (IP)
3 (FS)
4 (FM+)

b)


4
3
2
1
Size (x bill-length)
Meadow Pipit
Cuckoo
Nestling species

c)

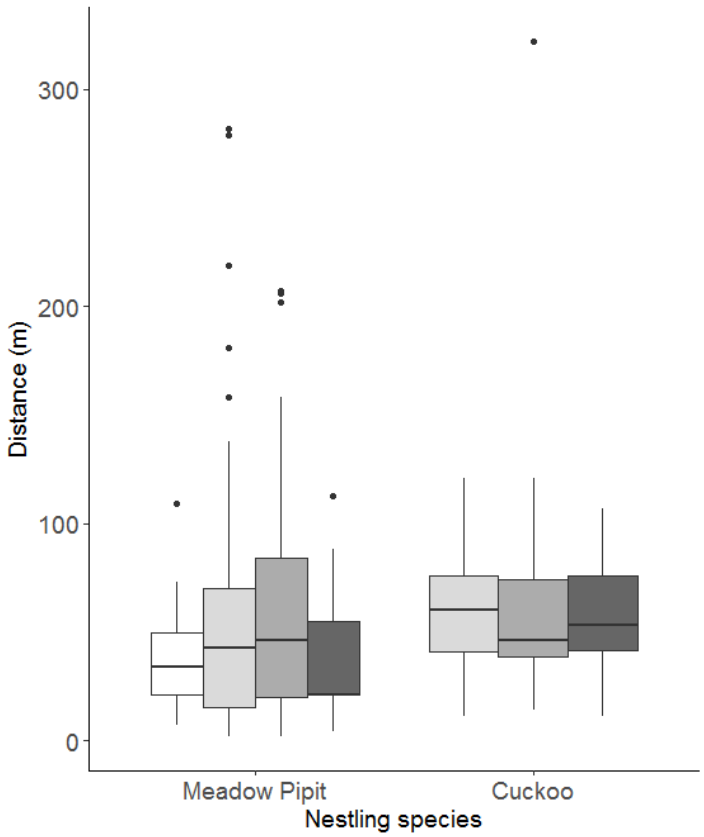


Figure 2. a) Provisioning visit rate, b) prey load size and c) foraging distance of pairs raising meadow pipit or cuckoo nestlings, segregated by feather growth stage CHICKSTAGE, NA (naked), IP (primaries in pin), FS (primaries up to 1/3 grown), FM+ (primaries over 1/3 grown)

observation session start time (START_HR) and with increasing mean bog cover scores (BO) on foraging plots. Provisioning visit rate also negatively correlated with observation session duration. Prey load size increased with nestling age, and was negatively associated with higher cloud cover (CLOUD) scores and bracken vegetation cover (BR) on foraging plots. Foraging distance significantly correlated with a range of foraging plot vegetation variables. Foraging plots containing more of either short (PG) or tall (HO) pastoral grasses or tufted semi-natural grassland grasses (TU) were significantly further from nests, while those containing low gorse (LG), bracken (BR), mosses (MO) heather (HE) or bare ground (BGRD) were significantly closer to nests (Table 2).

Fitting the above models to the full sample of 31 unparasitised and 6 cuckoo-parasitised nests, there was no additional significant effect of nestling species on the three provisioning behaviours, and it was in all cases excluded from the best candidate model during backward selection (Table 3) (Fig. 2). Provisioning visit rate was significantly higher in later nestling feather growth stages CHICKSTAGE (Fig. 2a), and was negatively associated with later observation session start time START_HR. Rate again negatively correlated with observation session length DURATION (Table 3a). Mean prey load size was negatively associated with greater cloud cover; and increasing mean bracken (BR) and heather (HE) cover scores on foraging plots (Table 3a). The final foraging distance model variables selected were foraging plot vegetation variables that largely reflected those in the final model for unparasitised nests only (Table 3b).

(iv) Comparing provisioning of pipit and cuckoo nestlings and cuckoo fledglings

There was a significantly higher provisioning visit rate for cuckoos during the post-fledging period, compared to cuckoos during the nestling stage and meadow pipit nestlings (Table 4) (Fig. 3a). This initial model, indicating an increase in provisioning visit rate in fledglings compared to nestlings and negative association of visit rate with time of observation (START_HR) and duration, was the best model (Table 4a). There was no significant difference in prey load size provisioned to cuckoos at the fledgling stage compared to their nestling stage or nestling meadow pipits (Table 4a) (Fig. 3b). Prey load size of nestling or fledgling cuckoos was negatively associated with foraging plot bracken cover (BR) (Table 4a). Foraging distances flown during provisioning of cuckoos during the post-fledging period were longer than during nesting cuckoo stages or when raising meadow pipit nestlings, though statistical significance was marginal (type III output from final model, YOUNG significance $P = 0.046$) (Table 4b) (Fig. 3c). Only 33% of foraging plot visits by pipits provisioning a fledgling cuckoo were made within 100 m of the fledgling (n=33, q1 = 67 m, median = 106 m, q3 = 322 m). Many foraging plots visited by birds provisioning fledgling cuckoos were foraging plots previously used during the nestling stage. Measuring distances from the original nest of foraging plots used during

Table 2. Estimates and standard errors (S.E.) of change in hourly provisioning visit rate, mean prey load size and foraging trip distance in response to variables in final generalized linear mixed models for a sample of unparasitised meadow pipit nests, plus probability values.

| Response | Model | | |

Table 3a. Estimates and standard errors (S.E.) of change in hourly provisioning visit rate and mean prey load size in response to variables in initial (from Table 2) and final generalized linear mixed models for a sample of cuckoo-parasitised (SPECIES = CK) and unparasitised meadow pipit nests, plus probability values.

	Rate (h^{-1}) (n = 36 nests)					
	Intercept +	CHICKSTAGE IP	FS	FM+	+ START_HR + DURATION + BO +	$SPECIES_{CK}$
Est.	32.15	+3.32 |	+5.05 |	+3.60	-0.56 -0.32 -3.62	-1.14
S.E.	3.76	1.47 |	1.49 |	1.97	0.18 0.04 1.83	1.52
P	<0.0001	0.028 |	0.001 |	0.073	0.003 <0.0001 0.052	0.46

	Intercept +	CHICKSTAGE IP	FS	FM+	+ START_HR + DURATION
Est.	31.07	+2.92 |	+4.23 |	+2.74	-0.50 -0.31
S.E.	3.71	1.49 |	1.47 |	1.86	0.18 0.04
P	<0.0001	0.054 |	0.005 |	0.144	0.007 <0.0001

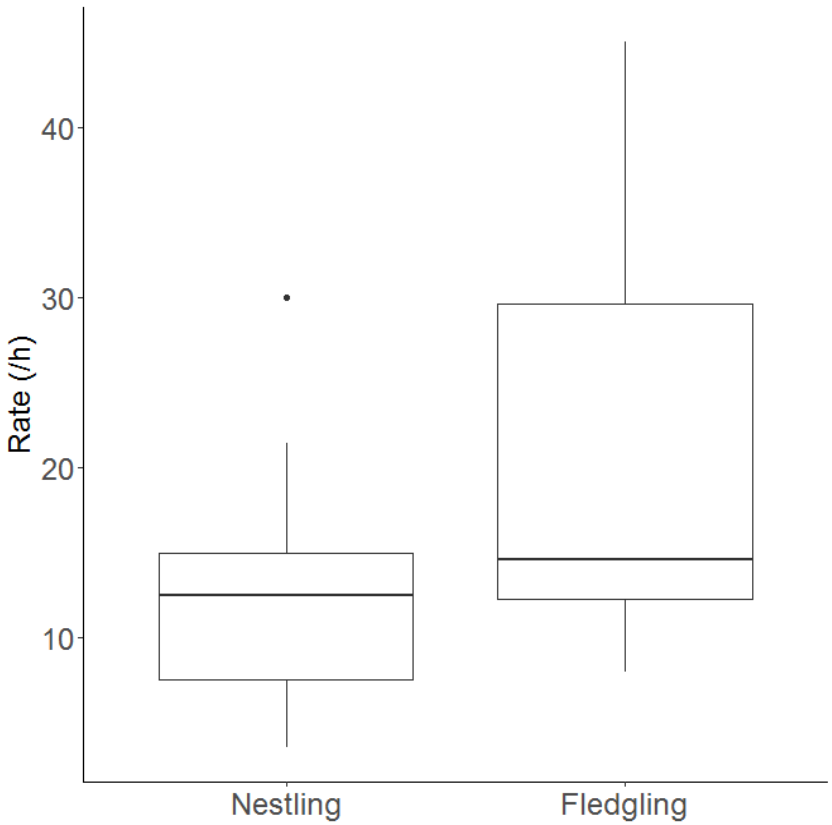
	Size (x bill length) (n = 32 nests)						
	Intercept +	CHICKSTAGE IP	FS	FM+	+ $CLOUD_{2,3}$ 33-67%	67-100%	+ BR + $SPECIES_{CK}$
Est.	1.740	+0.639 |	+0.765 |	+1.194	-0.824 |	-0.385	-0.210 -0.266
S.E.	0.382	0.291|	0.300 |	0.361	0.235 |	0.229	0.062 0.235
P	<0.0001	0.033 |	0.014 |	0.002	0.001 |	0.099	0.002 0.273

	Intercept +	$CLOUD_{2,3}$ 33-67%	67-100%	+ BR + HE
Est.	2.886	-0.958 |	-0.639	-0.237 -0.215
S.E.	0.281	0.235 |	0.220	0.055 0.089
P	<0.0001	0.0001|	0.005	<0.0001 0.019

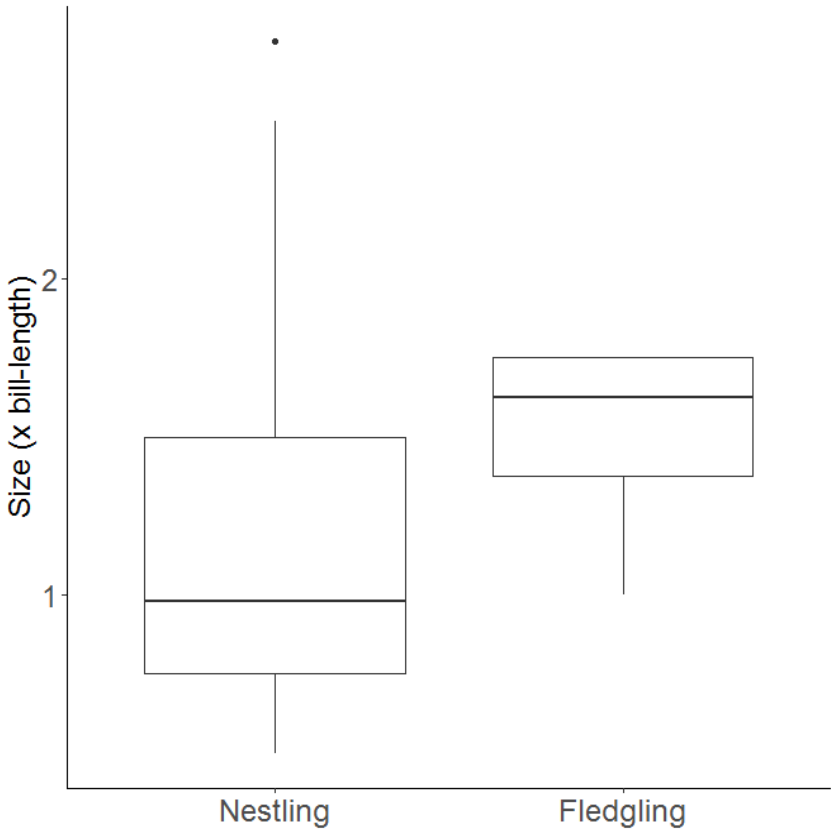
Table 3b. Estimates and standard errors (S.E.) of change in foraging trip distance in response to variables in initial (from Table 2) and final generalized linear mixed models for a sample of cuckoo-parasitised (SPECIES = CK) and unparasitised meadow pipit nests, plus probability values. Subscripts are factor levels; grey text is values/ranges they represent.

	Foraging distance (m) n = 36 nests									
	Int +	$LG_{1,2}$ +	PG_1 +	HO_1 +	$TU_{1,2,3,4}$ +	BR_{1-5} +	$HE_{1,2,3}$ +	$MO_{1,2,3}$ +	$BGRD_{1,2}$ +	SPECIES
		Pr-5 5-75	*25-100*	*75-100*	*Pr-5 5-25 25-50 50-100*	*present*	*Pr-5 5-25 25-100*	*Pr-5 5-25 25-100*	*<0-5 5-75*	*CK*
Est.	80.7	-11.0 | -41.3	+147.1	+95.7	+7.0|-7.5|+22.4|+7.9	< -24.2	-4.4 |-17.2 |-7.0	+8.6| -2.0| -29.3	+11.8|-10.3	+6.7
S.E.	12.2	4.7 | 7.2	14.4	24.0	7.1| 8.0| 10.0|10.0	<9.2	5.3 | 6.8 | 7.0	4.9 | 7.0 | 8.6	3.9 | 7.1	16.4
P	<0.0001	0.02 |<0.0001	<0.0001	<0.0001	0.3| 0.3| 0.03| 0.4	<0.001	0.4 | 0.01 | 0.3	0.08 | 0.8 | 0.0007	0.003|0.1	0.7
	Intercept +	$LG_{1,2}$ +	PG_1 +	HO_1 +	$TU_{1,2,3,4}$ +	BR_{1-5} +	$MO_{1,2,3}$ +	$BGRD_{1,2}$ +	$BI_{1,2}$	
		Pr-5 % 5-75	*25-100*	*75-100*	*Pr-5 5-25 25-50 50-100*	*present*	*Pr-5 5-25 25-100*	*<0-5 5-75*	*<0-5 5-100*	
Est.	73.9	-9.9 | -45.5	+158.1	+108.4	+5.2|-9.4|+19.6|+3.6	< -20.4	+14.4|+0.5 |-29.9	+10.5|-6.6	-14.7 |+10.7	
S.E.	11.2	4.5 | 6.9	13.8	23.3	6.9 | 7.6| 9.6 | 9.3	<8.4	4.9 | 6.9 | 8.1	3.8 | 6.7	4.5 | 8.4	
P	<0.0001	0.03 |<0.0001	<0.0001	<0.0001	0.5| 0.2| 0.04| 0.7	<0.008	0.004| 0.9 | 0.0003	0.006 | 0.3	0.001|0.2	

a)


40
30
20
10
Rate (/h)
Nestling
Fledgling

b)


2
1
Size (x bill-length)
Nestling
Fledgling

c)

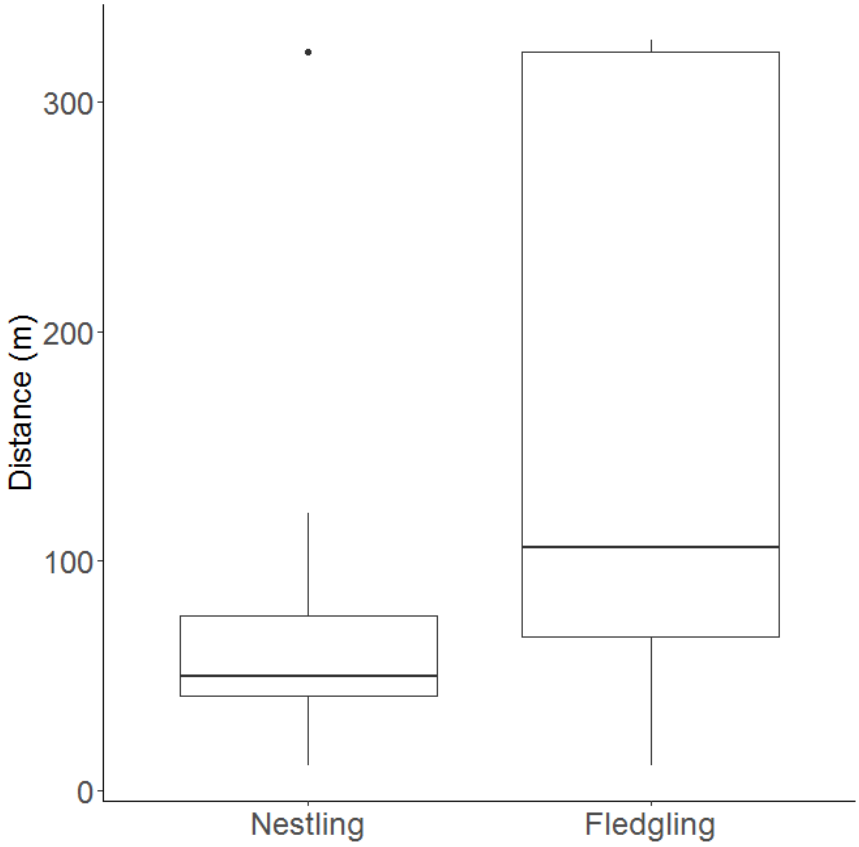


Figure 3. a) Provisioning visit rate, b) prey load size and c) foraging distance of meadow pipits provisioning a cuckoo nestling or fledgling.

fledgling provisioning, 42% of foraging plot visits were within 100 m of the original nest (n=33, q1 = 69 m, median = 150 m, q3 = 351 m).

(v) Comparing prey fed to meadow pipit nestlings and cuckoo nestlings or fledglings

The comparative prey analysis found 256 distinct COI DNA sequences that matched with ≥97% sequence similarity to Barcode of Life Database (BOLD) v4 species-level barcodes, and these sequences together accounted for 830133 reads across 19 MISEQ runs of 11 nestling/fledgling cuckoo faecal samples (n = 7 nestling samples from 4 nests, and 4 fledgling samples from one of these nests plus one further nest)

Table 4a. Estimates and standard errors (S.E.) of change in hourly provisioning visit rate and mean prey load size in response to variables in initial (from Table 2) and final generalized linear mixed models for a sample of meadow pipits provisioning meadow pipit nestlings, and cuckoo nestlings and fledglings (YOUNG = MP, CKn, CKf), plus probability values.

	Rate (h^{-1}) (n = 37 nests)				
	Intercept +	YOUNG (CKn CKf)	+ START_HR +	DURATION +	BO
Est.	39.91	-1.02 | +6.66	-0.67	-0.37	-2.68
S.E.	3.68	1.37 | 2.01	0.18	0.04	2.18
P	<0.0001	0.460 | 0.002	0.001	<0.0001	0.224
	Intercept +	YOUNG (CKn CKf)	+ START_HR +	DURATION	
Est.	39.10	-0.78 | +6.92	-0.64	-0.37	
S.E.	3.65	1.37 | 2.02	0.18	0.04	
P	<0.0001	0.571 | 0.001	0.001	<0.0001	
	Size (x bill length) (n = 33 nests)				
	Intercept +	YOUNG (CKn CKf)	+ $CLOUD_{2,3}$ (33-67% 67-100%)	+	BR
Est.	2.48	-0.01 |+0.22	-0.83 | -0.60		-0.20
S.E.	0.24	0.20 | 0.34	0.24 | 0.23		0.05
P	<0.0001	0.980 | 0.515	0.001 | 0.010		0.001
	Intercept +	$CLOUD_{2,3}$ (33-67% 67-100%)	+ BR +	HE	
Est.	2.81	-0.91 | -0.64	-0.21	-0.21	
S.E.	0.27	0.23 | 0.21	0.05	0.084	
P	<0.0001	0.0002 | 0.004	<0.0001	0.017	

Table 4b. Estimates and standard errors (S.E.) of change in foraging trip distance in response to variables in initial (from Table 2) and final generalized linear mixed models for a sample of meadow pipits provisioning meadow pipit nestlings, and cuckoo nestlings and fledglings (YOUNG = MP, CKn, CKf), plus probability values. Subscripts are factor levels; grey text is values/range they represent.

	Foraging distance (m) (n = 37 nests)									
	Intercept	+ $LG_{1,2}$	+ PG_1	+ HO_1	+ $TU_{1,2,3,4}$	+ BR_{1-5}	+ $HE_{1,2,3}$	+ $MO_{1,2,3}$	+ $BGRD_{1,2}$	+ YOUNG
		Pr-5% 5-75	*25-100*	*75-100*	*Pr-5 5-25 25-50 50-100*	*present*	*Pr-5 5-25 25-100*	*Pr-5 5-25 25-100*	*<0-5 5-75*	*CKn CKf*
Est.	77.0	-9.9 | -38.7	+165.2	+95.2	+8.3|-3.7|+24.7|+12.6	< -23.8	-4.8 |-19.6 |-7.5	+8.8| +1.4| -28.3	+12.9|-12.6	+6.2 | +35.3
S.E.	12.2	4.8 | 7.4	13.7	25.2	6.5| 7.4| 9.6 | 9.6	<9.4	5.4 | 6.7 | 7.1	5.0 | 6.8 | 8.7	3.8 | 7.3	16.1 | 17.7
P	<0.0001	0.04 |<0.0001	<0.0001	0.0002	0.2| 0.6| 0.01| 0.2	<0.002	0.4 | 0.004 | 0.3	0.08 | 0.8 | 0.001	0.0001|0.08	0.7 | 0.05
	Intercept	+ $LG_{1,2}$	+ PG_1	+ HO_1	+ TU_3	+ BR_{1-5}	+ $HE_{1,2,3}$	+ $MO_{1,2,3}$	+ $BGRD_{1,2}$	+ $BI_{1,2}$	+ YOUNG
		Pr-5% 5-75	*25-100*	*75-100*	*25-50*	*Present*	*Pr-5 5-25 25-100*	*Pr-5 5-25 25-100*	*<0-5 5-75*	*<0-5 5-100*	*CKn CKf*
Est.	77.6	-8.7 | -39.3	+168.9	+97.7	+26.5	<-22.7	+7.0|-19.0 |-10.5	+12.5|+1.6 |-31.3	+12.1|-12.6	-14.1 |+10.9	+8.5 |+32.2
S.E.	12.0	4.7 | 7.3	13.6	25.0	9.7	<9.2	5.4 | 6.6 | 7.0	5.1 | 7.0 | 8.7	3.8 | 7.1	4.5 | 8.8	16.2 | 17.9
P	<0.0001	0.07 |<0.0001	<0.0001	0.0001	0.007	<0.003	0.19 |0.004|0.14	0.015| 0.8 | 0.0004	0.001| 0.8	0.002 |0.2	0.6 | 0.08

and 18 MISEQ runs of 12 meadow pipit brood faecal samples (n = 12 nestling-stage brood samples from 12 nests).

For the baseline prey analysis, Sanger sequencing was carried out on a total of 861 clones of COI DNA from 81 pipit nestling faeces. 214 unique sequences matched with ≥98% similarity to one or more species-level barcode sequences in BOLD v4. Identification to order or lower taxonomy was achievable for 180 unique sequences read from study faecal samples. Cluster analysis showed these sequences matched to a total of 433 cloned sequences from 80 faecal samples. These represented 63 different meadow pipit broods across all four sampled sites. In the comparative analysis, prey orders found in faecal samples at more than 50% of both cuckoo-parasitised and unparasitised pipit nests were Diptera, Orthoptera (family Acrididae) and Lepidoptera. Additionally, Hymenoptera (family Tenthredinidae) were also detected at more than 50% of cuckoo-parasitised nests. Diptera were found in 100% of faecal samples from cuckoo young, and 58.3% of faecal samples from meadow pipit nestlings. Using a 5 mm adult wing length as the threshold (following Brooke & Davies 1989), DNA representing 'small' Diptera were found in 100% of cuckoo faecal samples, compared to 50% of meadow pipit samples and nests. Sequences representing large-size Diptera were found in samples covering 100% of cuckoo-parasitised nests (90.9% of samples), compared to 58.3% of meadow pipit broods (58.3% of samples) (Table 5). The most frequent order-level taxon by DNA in meadow pipit brood faeces was Orthoptera. There was statistically a significantly higher rate of occurrence of Chloropidae and Rhagionidae in prey fed to cuckoo nestlings or fledglings (binomial logistic regression for both taxa, n = 17 nests, modelled occurrence 0.0006 in meadow pipit nestling diet, 0.9999 in cuckoo nestling/fledgling diet, P = 0.00544). Occurrence of the orders Coleoptera, Orthoptera, Hymenoptera and Lepidoptera, and the families Muscidae, Tipulidae, Tenthrenidae, Lasiocampidae, Noctuidae, Oecophoridae and Geometridae did not significantly differ between nestling meadow pipit and nestling or fledgling cuckoo diets. A model was not fitted for occurrence of Diptera as this taxon occurred in 100% of cuckoo samples Operational taxonomic units (OTUs) representing 40 arthropod families were detected in DNA extracted from 11 cuckoo faecal samples, compared to 25 families in 12

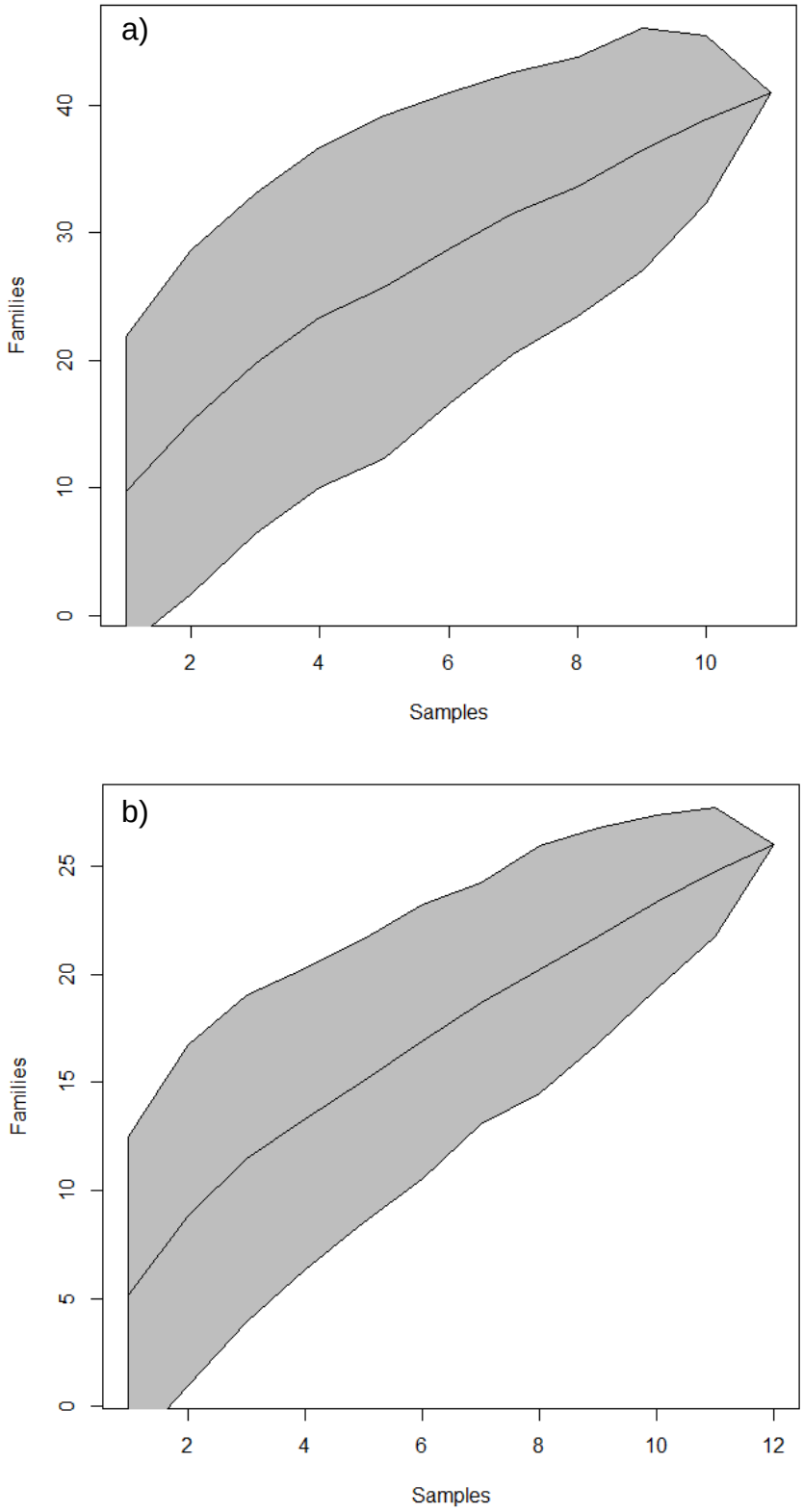


Figure 4. Accumulation curves of arthropod families detected by MISEQ sequencing of COI DNA from faecal samples of a) common cuckoo nestlings or fledglings and b) meadow pipit nestling broods.

Table 5. Frequency of DNA from different invertebrate orders, families and species in faecal samples from common cuckoo young or meadow pipit broods. Black: 'comparative analysis' MISEQ of samples from Holne Moor and Meldon Common 2016. Grey: 'baseline analysis' clone and Sanger sequencing of meadow pipit samples from four sites 2015-2017.

		Frequency (% of samples)			Frequency (% of nests)		
	Species	Cuckoo n = 11	Meadow Pipit n = 12	Meadow Pipit n = 80	Cuckoo n = 5	Meadow Pipit n = 12	Meadow Pipit n = 63
ARACHNIDA							
Araneae		**9.1**	**0.0**	**11.3**	**20.0**	**0.0**	**14.3**
Gnaphosidae	*Drassodes cupreus*	9.1	0.0	0.0	20.0	0.0	0.0
Tetragnathidae		9.1	0.0	5.0	20.0	0.0	6.3
	Metellina segmentata	9.1	0.0	0.0	20.0	0.0	0.0
	Pachygnatha degeeri	0.0	0.0	5.0	0.0	0.0	6.3
Thomisidae	*Xysticus erraticus*	0.0	0.0	6.3	0.0	0.0	7.9
Sarcoptiformes							
Chamobatidae	*Chamobates pusillus*	9.1	0.0	0.0	20.0	0.0	0.0
INSECTA							
Coleoptera		**18.2**	**8.3**	**10.0**	**40.0**	**8.3**	**12.7**
Cantharidae	*Cantharis pallida*	9.1	0.0	0.0	20.0	0.0	0.0
Carabidae	(unknown)	0.0	8.3	1.3	0.0	8.3	1.6
Dascillidae	*Dascillus cervinus*	0.0	0.0	1.3	0.0	0.0	1.6
Elateridae		0.0	0.0	5.0	0.0	0.0	6.3
	Actenicerus sjaelandicus	0.0	0.0	2.5	0.0	0.0	3.2
	Athous haemorrhoidalis	0.0	0.0	1.3	0.0	0.0	1.6
Staphylinidae	*Ocypus aeneocephalus*	9.1	0.0	2.5	20.0	0.0	3.2

Diptera		**100.0**	**58.3**	30.0	**100.0**	**58.3**	34.9
Large diptera >5mm wing		90.9	58.3	18.8	100.0	58.3	22.2
Small diptera <5mm wing		100.0	50.0	12.5	100.0	50.0	14.3
Anisopodidae	*Sylvicola stackelbergi*	9.1	0.0	0.0	20.0	0.0	0.0
Anthomyiidae		9.1	8.3	0.0	20.0	8.3	0.0
Asilidae	*Machimus atricapillus*	9.1	0.0	0.0	20.0	0.0	0.0
Bibionidae	*Dilophus febrilis*	0.0	8.3	8.8	0.0	8.3	9.5
Calliphoridae	*Lucilia sericata*	0.0	0.0	1.3	0.0	0.0	1.6
Cecidomyiidae	*Monardia toxicodendri*	9.1	0.0	0.0	20.0	0.0	0.0
Ceratopogonidae	*Serromyia sp.*	0.0	0.0	1.3	0.0	0.0	1.6
Chloropidae	*(total)*	90.9	41.7	0.0	100.0	41.7	0.0
	Tricimba sp.	27.3	0.0	0.0	40.0	0.0	0.0
Culicidae	*(total)*	9.1	8.3	0.0	20.0	8.3	0.0
Dolichopodidae	*(total)*	9.1	16.7	0.0	20.0	16.7	0.0
Ephydridae	*(total)*	9.1	0.0	0.0	20.0	0.0	0.0
Hybotidae	*Hybos grossipes*	9.1	0.0	1.3	20.0	0.0	1.6
Limoniidae	*Limonia nubeculosa*	0.0	0.0	1.3	0.0	0.0	1.6
Muscidae	*(total)*	27.3	8.3	1.3	60.0	8.3	1.6
Rhagionidae		90.9	41.7	7.5	100.0	41.7	9.5
	Rhagio scolopaceus	63.6	41.7	7.5	80.0	41.7	9.5
	Rhagio tringarius	27.3	0.0	0.0	40.0	0.0	0.0
	Rhagio lineola	9.1	0.0	0.0	20.0	0.0	0.0
Scathophagidae	*Scathophaga stercoraria*	9.1	0.0	2.5	20.0	0.0	1.6
Syrphidae	*(total)*	18.2	0.0	1.3	40.0	0.0	1.6
Tabanidae	*(unknown)*	9.1	8.3	2.5	20.0	8.3	3.2
Tachinidae	*(total)*	9.1	0.0	1.3	20.0	0.0	1.6

Tipulidae		45.5	25.0	3.8	60.0	25.0	4.8
	Tipula paludosa	36.4	25.0	1.3	60.0	25.0	1.6
	Tipula fascipennis	0.0	8.3	0.0	0.0	8.3	0.0
Hemiptera		**9.1**	**8.3**	**23.8**	**20.0**	**8.3**	**28.6**
Aphrophoridae	*Philaenus spumarius*	9.1	0.0	0.0	20.0	0.0	0.0
Cicadellidae	*Psammotettix confinis*	0.0	0.0	2.5	0.0	0.0	3.2
Miridae		0.0	8.3	22.5	0.0	8.3	27.0
Hymenoptera							
Braconidae	*Praon barbatum*	0.0	0.0	1.3	0.0	0.0	1.6
Tenthredinidae	*(unknown)*	45.5	8.3	0.0	60.0	8.3	0.0
Lepidoptera		**72.7**	**58.3**	**42.5**	**80.0**	**58.3**	**42.9**
Choreutidae	*Choreutis lutescens*	0.0	8.3	0.0	0.0	8.3	0.0
Crambidae		9.1	8.3	13.8	20.0	8.3	17.5
Depressariidae	*Depressaria leucocephala*	0.0	8.3	0.0	0.0	8.3	0.0
Erebidae	*(unknown)*	18.2	8.3	1.3	40.0	8.3	1.6
Gelechiidae	*Bryotropha terrella*	0.0	8.3	0.0	0.0	8.3	0.0
Geometridae		36.4	8.3	8.8	60.0	8.3	11.1
	Scotopteryx luridata	9.1	0.0		20.0	0.0	4.8
Glyphipterigidae	*Glyphipterix fuscoviridella*	18.2	8.3	3.8	40.0	8.3	3.2
Hesperiidae	*(unknown)*	9.1	0.0	0.0	20.0	0.0	0.0
Lasiocampidae	*(total)*	18.2	16.7	1.3	20.0	16.7	1.6
Lycaenidae	*(unknown)*	9.1	8.3	0.0	20.0	8.3	0.0
Momphidae	*(unknown)*	9.1	0.0	0.0	20.0	0.0	0.0
Noctuidae	*(total)*	27.3	16.7	6.3	40.0	16.7	7.9
	Aporophyla nigra	9.1	8.3	0.0	20.0	8.3	0.0

	Phlogophora meticulosa	18.2	8.3	0.0	20.0	8.3	0.0
	Autographa gamma	0.0	0.0	1.3	0.0	0.0	1.6
	Cerapteryx sp.	0.0	0.0	1.3	0.0	0.0	1.6
	Hypena proboscidalis	0.0	0.0	1.3	0.0	0.0	1.6
	Rusina ferruginea	0.0	0.0	1.3	0.0	0.0	1.6
	Xestia castanea	0.0	0.0	1.3	0.0	0.0	1.6
Notodontidae	*Elasmia schausi*	9.1	0.0	0.0	20.0	0.0	0.0
Nymphalidae	*(unknown)*	18.2	0.0	0.0	20.0	0.0	0.0
Oecophoridae	*(total)*	54.5	50.0	5.0	60.0	50.0	6.3
Thyrididae	*(unknown)*	9.1	8.3	0.0	20.0	8.3	0.0
Tortricidae	*(unknown)*	27.3	0.0	1.3	40.0	0.0	1.6
Ypsolophidae	(unknown)	0.0	0.0	2.5	0.0	0.0	3.2
Mecoptera							
Panorpidae	*Panorpa germanica*	9.1	0.0	0.0	20.0	0.0	0.0
Orthoptera							
Acrididae	*Omocestus sp.*	36.4	83.3	0.0	60.0	83.3	0.0
Trichoptera							
Odontoceridae	*Odontocerum albicorne*	9.1	0.0	0.0	20.0	0.0	0.0
Ephemeroptera							
Baetidae	*Baetis niger*	0.0	0.0	1.3	0.0	0.0	1.6
Plecoptera							
Leuctricidae	*Leuctra sp.*	0.0	0.0	1.3	0.0	0.0	1.6
[COLLEMBOLA]							
Entomobryomorpha		**0.0**	**0.0**	2.5	**0.0**	**0.0**	3.2
Entomobryidae	*Entomobrya sp.*	0.0	0.0	1.3	0.0	0.0	1.6
Tomoceridae	*Tomocerus longicornis*	0.0	0.0	1.3	0.0	0.0	1.6

MALACOSTRACA							
Isopoda							
Oniscidae	*Oniscus asellus*	**0.0**	**0.0**	15.0	**0.0**	**0.0**	17.5
DIPLOPODA							
Julida							
Julidae	*Julus scandinavius*	**0.0**	**0.0**	1.3	**0.0**	**0.0**	1.6
CLITELLATA							
Haplotaxida							
Lumbricidae	*Lumbricus rubellus L1*	**0.0**	**0.0**	26.3	**0.0**	**0.0**	31.7

meadow pipit brood samples (chi-square goodness of fit test $\chi^2 = 4.898$, df = 1, *P* = 0.0269). Rarefaction using the total MISEQ reads of COI sequences representing each identified arthropod family, estimated the diversity of families detected per 100000 reads to be 37.42 across cuckoo faecal samples and 25.28 across meadow pipit brood faecal samples. The accumulation curve for families detected in successive faecal samples showed reduced plateau for pipit nestlings than cuckoo nestlings or fledglings (Fig. 4). While overall invertebrate diet diversity was higher for cuckoo nestlings, diversity indices (using the number of MISEQ reads per taxonomic family per sample) suggested individual meadow pipit brood faecal samples had greater invertebrate diversity than individual cuckoo faecal samples. The exponent of the median Shannon diversity index across meadow pipit faecal samples was 2.645 (or $e^{0.9725}$), compared to 2.232 (or $e^{0.8031}$) across cuckoo faecal samples. Across the baseline and comparative analyses, 48 families were detected in meadow pipit brood faeces (Table 5).

Across the comparative and baseline analyses, orders Sarcoptiformes (mites), Mecoptera and Trichoptera were detected only in cuckoo faeces. Orders Ephemeroptera (mayflies), Plecoptera (stoneflies), Entomobryomorpha (springtails) and Julida (millipedes) were detected only in meadow pipit brood faecal samples. All of the above were rare in occurrence. However, orders Haplotaxida (specifically earthworms Lumbricidae) and Isopoda (specifically woodlice Oniscidae) were detected only in the baseline analysis from Sanger sequencing of meadow pipit brood faeces, and occurred in more than 30% and 15% of nests, respectively.

From the baseline analysis alone, the most prevalent taxa (per nest) provisioned to meadow pipit broods were insect orders Lepidoptera (42.9%), Diptera (34.9%), and Hemiptera (28.6%); and annelid order Haplotaxida (earthworms Lumbricidae, 31.7%) (Table 5). In particular for Lepidoptera, Diptera and Coleoptera, constituent families or species provisioned to pipit nestlings were diverse and largely each occurred at low frequency. However, the lepidopteran family Crambidae was represented heavily by one species *Chrysoteuchia culmella*. Hemiptera, Araneae and Haplotaxida were similarly represented mainly by one or two species.

(vi) Nest failure rates of parasitised and unparasitised nests

Nests containing a cuckoo nestling had significantly higher daily probability of nest failure. This was the case both for all nests monitored on site that reached the nestling stage (GLMM logistic binomial n = 145 nests, intercept est. (logit) = -2.16 ± 0.19, SPECIES est. (logit) = +1.95 ± 0.82 for cuckoo, probability of failure ~ 0.448 for cuckoo c.f. ~0.103 for unparasitized nests. P = 0.0173), and for only nests that survived to the point of receiving provisioning observation, similarly to those analysed in chapter 3 (GLMM logistic binomial n = 47 nests, intercept estimate (logit) = -13.56 ± S.E. 2.21, SPECIES est. (logit) = +23.27 ± 5.22, modelled probability of failure ~0.9999 c.f. 1.29 x 10-6 for unparasitized nests. $P < 0.0001$).

Discussion

i) Comparing vegetation associations of parasitised and non parasitised nests

There was significant variation in occurrence of cuckoo parasitism with vegetation cover around meadow pipit nests. Parasitised nests had lower mean cover of tufted semi-natural grassland grasses and *Juncus* rushes. These are tall and dense vegetation present throughout the season, within which watching and locating host nests may be more difficult for cuckoos. Few nest visits by female cuckoos to lay eggs were observed at the site but some involved several minutes on the ground seemingly locating or re-locating the nest cup and at least one targeted nest was not successfully parasitised (also noted for meadow pipit nests in Chance 1940 and Davis 1996). This suggests precise nest location is not always known by female cuckoos in advance and vegetation could influence parasitism success. Occurrence of cuckoo parasitism was higher for nests within 100 m of water. This may be due to water bodies on site being associated with low ground in the landscape, which may be easier for cuckoos to watch to find nests. Topography around nests was not directly measured by the present study. The importance of visibility to cuckoo nest parasitism success or rate has been demonstrated in previous studies (Alverez 1993, Øien *et al.* 1996, Clarke *et al.* 2001) but was associated with tree height

Figure 5. Photograph illustrating mimicry of dead bracken *Pteridium aquilinum* by cuckoo juvenile plumage. Image by Charles R Tyler.

or presence rather than topography: The present study did not find that tree presence predicted cuckoo parasitism. The focal habitats of past studies have generally been reed-bed (Øien *et al.* 1996, Clarke *et al.* 2001), but with at least one in dry wooded habitats (Alverez 1993). These are mostly flatter than the terrain of the present study site. Additional visibility afforded to cuckoos by presence or heights of trees may therefore be less consistent across upland breeding areas.

Mean bracken cover was positively associated with nest parasitism. Peak egg laying periods of cuckoos in meadow pipit nests are mid-May and early June according to nest record card data (Rose 1982), which is earlier than growth and maturation of bracken takes place (mid to late June). Cuckoos may select host nests with on average greater extent of bracken, for the cover it provides during the later nestling and fledgling stages in subsequent weeks, with immature cover during peak laying having no negative effect on nest finding as with tall grasses and *Juncus* rushes above. Juvenile plumage of cuckoos shows exceptional mimicry in colour and pattern with dead bracken litter (Fig. 5), the previous season's growth that during cuckoo nestling periods lies as a blanket of broken material at ground level including around nests (pers. obs.). At the study site ground cover of dead bracken material significantly correlates with live bracken cover (Pearson's r = 0.67, Spearman's rank correlation P <

0.0001). Bracken cover was previously modelled to be negatively associated with provisioning visit rate and prey load size (Chapter 3, Table 4), so influence is unlikely to relate to favourable host foraging. Difference in vegetation around parasitised nests may in fact relate to general habitat preference of the adult cuckoo. Principal component analysis suggested that no modelled principal component (PC) accounted for more than 25% of total variation in habitat around nests, and that parasitised nests did not occupy a distinct range of values for PC1 and PC2 than unparasitised nests, although a subset of parasitised nests formed a cluster (appendix 8). This difference in conclusion highlights that differences detected in modelling via the present study's backward selection approach are to be treated with caution due to the difference in sample size between parasitised and unparasitised meadow pipit nests, and that a subset of parasitised nests of similar habitat type (potentially as a result of one or a few individual female cuckoos' egg-laying locations) may have influenced statistical outcomes to suggest a broader trend. It was not possible in the present study to identify maternity of each cuckoo egg and nestling, but it would be of clear value in any future study.

(ii) Comparing foraging habitat selection of pipits raising cuckoo or own brood

Both parasitised and unparasitised pipit pairs preferred short semi-natural grassland grasses but avoided gorse, bracken, *Juncus* rushes, shrubs, trees, bare rock and water on foraging plots (Table 1). While some vegetation types were avoided by pairs raising their own young, and not significantly avoided by pairs raising a cuckoo (for example dead gorse stalks DG or bare ground BGRD), suggesting potentially reduced selectivity for certain vegetation types when parasitised, there was no habitat that was preferred by parasitised pipits and avoided by unparasitised pipits, or vice versa. Habitat preference of brood parasite hosts, and possible shifts due to parasite nestling presence, has not to our knowledge been previously examined. From a conservation perspective, this analysis indicates that little significant change in meadow pipit habitat selection takes place when raising a cuckoo nestling compared to raising host broods.

(iii) Comparing provisioning of cuckoo nestlings and host broods

Data collection from parasitised nests involved repeat observations and sampling of a relatively small number of cuckoo young, only some of which were studied in both nestling and post-fledging stages. On this basis, it is difficult to assess whether the low statistical significance of some differences in provisioning between parasitised and unparasitised nests was due to minimal effects of parasitism on provisioning behaviour or the lower number of observations of parasitised nests.

Provisioning visit rate, prey load size and foraging trip distance did not significantly differ between parasitised and unparasitised pipit pairs (Table 3). The result for visit rate is concurrent with some studies of other cuckoo-host pairings (Brooke & Davies 1989, Kilner *et al.* 1999). The study's finding of no significant difference in prey load size also concurs with past work (Brooke & Davies 1989), and absence of significant difference in rate and prey load together is effectively concurrent with Martín-Gálvez *et al.* (2005). Length of individual prey items fed to cuckoos is reported to be larger (Martín-Gálvez *et al.* 2005) and smaller (Grim & Honza 2001) in different studies, but the greater observer distance during provisioning meant the present study could not assess this. Graphing prey load sizes fed to cuckoo and meadow pipit nestlings against feather growth stage (Fig. 2b) suggested linear increase in prey load size for host pipit broods, and a peaked relationship with development stage for young cuckoos. These respective patterns strongly reflect previous data that used prey item size and nestling mass (Grim & Honza 2001). However, relationships of these respective model fits were not supported statistically in the present study, possibly in part due to limited data from parasitised nests. Sample size of prey load data was the most limited of the three measurements of provisioning in the present study, and measurement success largely depended on successful observation via telescope of returning birds, which did not always perch in view before attending the nest. It is recommended that this study's field observation methodology is combined with cameras capturing continuous video at close proximity for the duration of observation sessions. Analysis of relative foraging distance undertaken by pairs hosting a cuckoo is not previously reported in the literature, but is essential for determining whether pairs which provision a

cuckoo nestling with similar visit rates and prey load sizes were maintaining this through shorter foraging distances. The present study suggested that host pairs did not maintain food provisioning rate when raising the nestling cuckoo as opposed to a brood through foraging nearer or further from the nest.

There were few habitat effects on provisioning behaviour and these did not differ between parasitised and unparasitised nests (Table 2, Table 3), suggesting that habitat had a similar influence on provisioning between parasitised and unparasitised nests (analysis (ii)).

(iv) Comparing provisioning of pipit and cuckoo nestlings and cuckoo fledglings

Cuckoos were provisioned at a higher visit rate as fledglings than as nestlings, but there was no significant modelled difference in prey load size between nestling and fledgling stage of individuals (Table 4a). This suggests that fledglings are provisioned more food per hour than nestlings, as expected from the greater size and energetic requirement for growth. Food brought per hour was previously measured to show linear increase with mass in cuckoo young (Grim & Honza 2001), but the only study of post-fledging changes in body size suggests that mass does not increase after fledging, with investment primarily in flight feather growth (Tyller *et al.* 2018). The post-fledging period of the common cuckoo is one of the least studied stages of the breeding cycle and data on fledgling provisioning rate is limited to studies in two hosts, common chaffinch *Fringilla coelebs* (Tyller *et al.* 2018) and common redstart *Pheonicurus phoenicurus* (Kysucan *et al.* in press). No study is known within the literature that quantitatively compares provisioning rate before and after fledging. One element reported from fledgling observations of *Cuculus spp.* and other parasites is that, in this phase, the cuckoo may receive food deliveries from additional birds than the original foster pair (Wyllie 1981, Sealy and Lorenzana 1997). This is most noticeable when the feeds are carried out by birds of a different species. Because pipits are sexually monomorphic and individuals were not marked, identifying further birds was not possible and it cannot be ruled out that extra-pair provisioning contributed to the higher provisioning rate measured in the fledgling stage. Pipits flew longer foraging distances while provisioning fledgling cuckoos than either nestling cuckoos or meadow pipits.

This included meadow pipits making use of foraging plots previously used during the cuckoo nestling stage that were in vicinity of the nest, even after the cuckoo fledgling had moved some distance from the nest. Longer foraging distances indicate greater energy investment per provisioning trip after cuckoo fledging. The nestling and fledgling periods of the cuckoo are also longer than those of host broods (Wyllie 1981, and observed in the present study) therefore the absolute energy investment of raising a cuckoo to independence is indicated to be greater than that for raising a host brood.

Fledgling meadow pipits could not be practically observed in the same way as fledgling cuckoos due to the larger number of birds, often moving under low vegetation. It is not clear whether broods overall receive similarly increased provisioning visit rates and foraging distances after fledging, and would require detailed tracking to observe.

(v) Comparing prey fed to nestling meadow pipits and cuckoo young

This study is the first to compare nestling prey between cuckoo and meadow pipit host nestlings, and to use a molecular DNA approach to identify cuckoo prey. A significantly larger number of invertebrate families were found in cuckoo faecal samples. This supports the suggestion by Grim and Honza (2001) that host pairs raising a cuckoo (or abnormally large host brood) may forage less selectively than pairs raising a host brood of typical size, due to larger parental effort required. An alternative mechanism for differing food composition in cuckoo nestlings is that host pairs raising a cuckoo are equally selective, but for different (perhaps more abundant but lower quality) prey. It is notable that individual meadow pipit faecal samples typically had higher Shannon diversity indices than individual cuckoo faecal samples. This supports the latter alternative mechanism. However, cuckoo faecal samples may consistently reflect a different duration or number of provisioning instances than those of meadow pipits, so data relating to within-sample diversity must be interpreted with caution. DNA approaches to diet analysis can detect prey even in trace presence and enable fine taxonomic identification, but they detect prey indirectly through DNA sequence matching and therefore give no indication of

prey item size or life stage (e.g. egg, larval, adult). However, Diptera families of all body sizes were detected with greater frequency in cuckoo faecal samples than in meadow pipit brood samples (Table 5). High occurrence of both size classes was previously recorded in cuckoos at reed warbler nests (Brooke & Davies 1989). Most nestling prey taxa were foliar arthropods or otherwise invertebrates likely to be found above the soil surface (Table 5). This follows our finding that short (mostly grazed) areas of grasses were selected by pipits but simple substrates that were barren such as bare earth and rock were not (Table 1).

Across the baseline and comparative analysis of meadow pipit faecal samples, high frequency of Lepidoptera and Diptera is consistent with some studies of meadow pipit nestling prey in UK uplands and lowlands (Walton 1979, Wilson *et al.* 1996, Douglas *et al.* 2008). Lepidoptera (larvae) are also key prey in European lowlands (van Klink *et al.* 2014). However, Tipulid larvae, often cited as a key dipteran prey of meadow pipits (Wilson *et al.* 1996, Evans *et al.* 2005, Hågvar *et al.* 2009), were not frequent. The array of dipteran and lepidopteran taxa detected were diverse and occurred at generally low frequencies. This follows past discussion that meadow pipits provision young with a wide range of invertebrates (Walton 1979), but with some selection shown among those in greatest abundance, possibly towards larger prey (Douglas *et al.* 2008, van Klink *et al.* 2014). In the baseline analysis, earthworms Haplotaxida were the third most prevalent prey taxon, while few previous studies have reported importance or even presence of earthworms in meadow pipit prey. Annelids are previously reported from lowland breeding meadow pipit diet, though not significantly relative to abundance (Wilson *et al.* 1996). Earthworms are reported as common prey in general across moorland bird species, although some species are rare or absent from peaty soils (Buchanan *et al.* 2006). Hemiptera were also frequent (Table 5). Hemipterans are important prey (i.e. relative to availability) in lowland farmland meadow pipit populations (Wilson *et al.* 1996) and they are common as prey across moorland birds in general (Buchanan *et al.* 2006). I also detected similar frequency of isopods (here woodlice *Oniscus asellus*) previously reported in the diet in lowland farmland (Wilson *et al.* 1996).

DNA of earthworms and isopod crustaceans (woodlice) were detected in the baseline meadow pipit prey analysis but not in the comparative analysis, and conversely Orthoptera DNA was detected in the comparative analysis but not in the baseline analysis. The high frequency of occurrence of these sequences in their respective datasets suggests different taxonomic sensitivity between the two sequencing protocols (clone and sequence versus Illumina MISEQ). Frequency of prey occurrence in the baseline study, sequencing 10 clones per faecal sample, did not surpass 50% while Illumina sequencing in the comparative study recorded frequency of 100% for some taxa (Table 5). The former method is limited by number of clones retrieved and sequenced per sample and therefore involves 'competition' between sequences for detection.

Prey composition from visual studies of remains in avian faeces is usually measured both in terms of frequency and dominance. Frequency measures the proportion of samples (or here, nests) in which a prey taxon was detected. Dominance considers what proportion of all prey bodies found across all samples is attributable to each prey taxon. Varying dominance and resultant biomass of prey may be the most critical factor in prey consumed or provisioned to developing nestlings. There are difficulties in transferring the measurement of dominance to molecular-based analysis of faeces, as a consistent number of sequences is not present that reflects number of individuals, due to variation in number of DNA copies per mitochondrion, mitochondria per cells and cells per organism. Conversely, frequency is a suitable measurement in DNA-based studies, because similarly to some visual studies the data is presence only rather than presence-absence. Compared to previous studies of frequency of invertebrate taxa in cuckoo nestling diet (Brooke & Davies 1989, Grim & Honza 2001), few taxa show similar position across studies when ranked from largest to smallest frequency, but in these two studies and the present study Diptera was of greatest frequency in all of them (91% in Brooke & Davies 1989, 96% in Grim & Honza 2001, 100% in present study). While some sequencing technology is geared towards maximum capacity to detect all traces of DNA in samples, others (such as the clone and sequence approach of the baseline analysis in this study) are directly limited in how many sequences can be captured, and in all cases it is not recommended to record no detection of a

sequence as absence of the relevant taxon. While in visual studies taxa are more likely to go undetected if they are soft-bodied or occur in actually low abundance; taxa in molecular analysis could occur in high actual abundance but go undetected due to use of PCR primers that do not target and amplify their DNA. The risk of this was minimised in the present study by preliminary testing of amplification of a range of invertebrates with selected PCR primers (chapter 2). While our range of test invertebrates was informed by past literature on pipit diet in a range of habitats, and selected primers are deemed suitable for amplifying DNA from a broad range of taxa, there remains some risk of failure to amplify outgroup taxa or a minority of taxa within the predicted target groups.

DNA yield was lower than expected upon extraction from faecal samples stored as described in this study (often below 1 ng μl^{-1} in 50 µl elution volume). This was expected to relate firstly to use of 80-90% ethanol to preserve samples, and secondly to decanting and air drying for removal of ethanol before extraction, as opposed to freeze drying. Together these could result in DNA entering solution from samples, which is then lost during decanting. I therefore recommend storage in 100% ethanol, freeze drying to remove ethanol, and storage at -80 °C between drying and the start of the extraction process (addition of lysis buffer). While some studies specify refrigerated storage temperature from the outset, as well as ethanol e.g. -20 °C (Trevelline *et al.* 2018), others successfully extracted DNA from samples stored at room temperature.

(vi) Nest survival rates of parasitised and unparasitised nests

Daily probability of nestling stage failure was significantly higher (around 3 times more likely) for nests containing a cuckoo. Probability of failure of meadow pipit nests (having reached the nestling stage and received provisioning observation) was previously shown not to statistically vary with nest provisioning visit rate, mean prey load size or mean foraging distance (chapter 3). Repetition with both parasitised and unparasitised nests at this specific stage also indicated no correlation with provisioning behaviour. Causes of failure at parasitised nests comprised predation or the nestling otherwise being located prematurely

outside the nest cup (pers. obs.). While some unparasitised broods died in the nest, generally in poor weather conditions and most probably associated with insufficient food and shelter, this was not noted among our (smaller) sample of parasitised nests. Overall the results indicate that daily nest failure rate (mainly from predation) is more likely for cuckoo chicks than for pipit broods, and this cannot be explained by different provisioning behaviour by pipit foster parents. Lower daily survival rate has been previously reported in nestling cuckoo relative to host broods (Sklepowicz & Halupka 2009). In that study it was suggested parasitised nests are easier to find both for brood parasites and nest predators. Avian nest predators such as corvids may watch nests and adult activity in a very similar manner to cuckoo females, and occupy the same perches as each other in the study area (pers. obs.). Cuckoo parasitism rates have previously been found to be inversely associated with qualitative measures of concealment from view from nearest trees (Øien *et al.* 1996). Concealment was not measured in the present study but is a valuable area of field data collection in cuckoo breeding studies. Bracken cover was positively correlated with cuckoo parasitism of nests, and reaches maturity at later stages of the cuckoo breeding season where dense cover may aid concealment of large nestlings, whose plumage also mimics dead bracken.

Conclusions

The aim of this study was to assess whether differences in nest habitat, provisioning and foraging of parasitised host pairs in a relative stronghold cuckoo habitat suggested that cuckoo nestling success required additional (foster) parental effort or habitat resources to those of host broods. Territory (100 m radius) habitat around parasitised nests was different, indicating selection or differential success between vegetation types by female cuckoos while parasitising nests. There were few differences in provisioning behaviour to suggest pairs hosting a cuckoo nestling make use of significantly different habitats or invest greater effort during the nestling stage, to raising a brood of their own young. However, fledgling cuckoos received prey at a greater rate with longer foraging distances, and prey fed to cuckoos was more diverse

throughout their development, suggesting additional invertebrate prey taxa are required to raise a cuckoo to independence. While cuckoos continue to return and young successfully fledge in upland semi-natural grasslands, food resources were not upland speciality taxa *per se*, and included taxa previously reported as common meadow pipit prey in lowland agricultural grasslands where intensive land management practices have had negative impacts on many invertebrate taxa (Vickery *et al.* 2001). The present study suggests cuckoo nestlings have different resource requirements to meadow pipits. Cuckoo breeding success requires meadow pipits that can invest greater absolute energy to the breeding attempt than is invested in a host brood, and can at later stages also expend energy at a greater rate per foraging trip than with nestling cuckoos or host broods. Greater diversity of available prey may be important for hosts to select specific alternative prey for a larger nestling (as is presented when raising a cuckoo), or successfully obtaining prey when normal prey selection is abandoned due to larger cuckoo growth requirements. These are not mutually exclusive. Conserving invertebrate biodiversity in cuckoo and meadow pipit habitat may be beneficial to conservation of breeding populations of both species.

References

Alverez F (1993) Proximity of trees facilitates parasitism by Cuckoos *Cuculus canorus* on Rufous Warblers *Cercotrichas galactotes*. *Ibis* 135: 331–331.

Antonov, A., Stokke, B. G., Moksnes, A., and Røskaft, E. (2007) First evidence of regular common cuckoo, *Cuculus canorus*, parasitism on eastern olivaceous warblers, *Hippolais pallida elaeica*. *Naturwissenschaften* 94(4): 307–312.

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67(1): 1–48.

Bibby, C. J., and Thomas, D. K. (1985) Breeding and diets of the Reed Warbler at a rich and a poor site. *Bird Study* 32(1): 19–31.

Birding Beijing (2018) The Beijing Cuckoo Project. *Birding Beijing*. Accessed: <https://birdingbeijing.com/beijing-cuckoo-project/. >.

Bishton, G. (1986) The diet and foraging behaviour of the Dunnock *Prunella modularis* in a hedgerow habitat. *Bird Study* 128: 526–539.

Bishton, G. (2001) Social structure, habitat use and breeding biology of hedgerow Dunnocks *Prunella modularis*. *Bird Study* 48(2): 188–193.

Braun-Blanquet, J. (1932) *Plant sociology. The study of plant communities.* New York: McGraw-Hill.

Brooke, M. D. L., and Davies, N. B. (1989) Provisioning of nestling cuckoos Cuculus canorus by reed warbler *Acrocephalus scirpaceus* hosts. *Ibis* 131(2): 250–256.

Brown, D. S., Jarman, S. N., and Symondson, W. O. C. (2012) Pyrosequencing of prey DNA in reptile faeces: analysis of earthworm consumption by slow worms. *Molecular Ecology Resources* 12(2): 259–266.

Brown, W.M. (1985). The mitochondrial genome of animals. In R.J. Maclntyre (ed.): *Molecular Evolutionary Genetics*, New York: Plenum Press, 95-130.

Buchanan, G. M., Grant, M. C., Sanderson, R. a., and Pearce-Higgins, J. W. (2006) The contribution of invertebrate taxa to moorland bird diets and the potential implications of land-use management. *Ibis* 148(4): 615–628.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13: 581–583.

Catchpole, C. K. (1972) A comparative study of territory in the Reed warbler (*Acrocephalus scirpaceus*) and Sedge warbler (*A. schoenobaenus*). *Journal of Zoology* 166(2): 213–231.

Chance, E. P. (1940) *The truth about the cuckoo*. London: Country Life.

Clare, E. L., Barber, B. R., Sweeney, B. W., Hebert, P. D. N., and Fenton, M. B. (2011). Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Molecular Ecology* 20(8): 1772-1780.

Clarke, A. L., Øien, I. J., Honza, M., Moksnes, A., and Røskaft, E. (2001) Factors Affecting Reed Warbler Risk of Brood Parasitism by the Common Cuckoo. *The Auk* 118(2): 534–538.

Cox, W. A., Thompson, F. R., Cox, A. S., and Faaborg, J. (2014) Post-fledging survival in passerine birds and the value of post-fledging studies to conservation. *Journal of Wildlife Management* 78(2): 183–193.

Davies, N. B., Kilner, R. M., and Noble, D. G. (1998) Nestling cuckoos, Cuculus canorus, exploit hosts with begging calls that mimic a brood. *Proceedings of the Royal Society B: Biological Sciences* 265(1397): 673–678.Davis, P. R. K. (1996) *The cuckoo on Exmoor and its meadow pipit host*. Exmoor Natural History Society.

Denerley, C., Redpath, S. M., van der Wal, R., Newson, S. E., Chapman, J. W., and Wilson, J. D. (2018) Breeding ground correlates of the distribution and decline of the Common Cuckoo *Cuculus canorus* at two spatial scales. *Ibis*. doi:10.1111/ibi.12612.

Douglas, D. J. T., Evans, D. M., and Redpath, S. M. (2008) Selection of foraging habitat and nestling diet by Meadow Pipits *Anthus pratensis* breeding on intensively grazed moorland. *Bird Study* 55(3): 290–296.

Douglas, D. J. T., Newson, S. E., Leech, D. I., Noble, D. G., and Robinson, R. a. (2010) How important are climate-induced changes in host availability for population processes in an obligate brood parasite, the European cuckoo? *Oikos* 119(11): 1834–1840.

Evans, D. M., Redpath, S. M., and Evans, S. A. (2005) Seasonal Patterns in the Productivity of Meadow Pipits in the Uplands of Scotland. *J. Field Ornithol.* 76(3): 245–251.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from

diverse metazoan invertebrates. *Molecular marine biology and biotechnology* 3: 294–299.

Glue, D., and Morgan, R. (1972) Cuckoo Hosts in British Habitats. *Bird Study* 19(4): 187–192.

Grim, T., and Honza, M. (2001) Does supernormal stimulus influence parental behaviour of the cuckoo's host? *Behavioral Ecology and Sociobiology* 49(4): 322–329.

Grim, T., and Honza, M. (1997) Differences in parental care of reed warbler (*Acrocephalus scirpaceus*) to its own nestlings and parasitic cuckoo (*Cuculus canorus*) chicks. *Folia Zoologica* 46: 135–142.

Grim, T., and Samaš, P. (2016). Growth performance of nestling cuckoos *Cuculus canorus* in cavity nesting hosts. *Acta Ornithologica* 51(2): 175-188.

Grim, T., Samaš, P., Procházka, P., Rutila, J., and Procházka, P. (2014) Are tits really unsuitable hosts for the Common Cuckoo? *Ornis Fennica* 91: 166–177.

Grim, T., Tyller, Z., and Samaš, P. (2017) Unusual diet of brood parasitic nestlings and its fitness consequences. *The Auk* 134(3): 732–750.

Hågvar, S., Glesne, O., and Østbye, E. (2009) Food habits and niche overlap in three alpine passerine birds, South Norway. *Ornis Norvegica* 32: 56–73.

Harris, S. J., Massimino, D., Gillings, S., Eaton, M. A., Noble, D. G., Balmer, D. E., Procter, D., and Pearce-Higgins, J.W. Woodcock, P. (2018) *The Breeding Bird Survey 2017. BTO Research Report 706*. Thetford.

Hebert, P. D. N., Cywinska, A., Ball, S. L., DeWaard, J. R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B - Biological Sciences* 270: 313–321.

Hewson, C. M., Thorup, K., Pearce-Higgins, J. W., and Atkinson, P. W. (2016) Population decline is linked to migration route in the Common Cuckoo. *Nature Communications* 7: 12296.

Kilner, R. M., Noble, D. G., and Davies, N. B. (1999) Signals of need in parent-offspring communication and their exploitation by the common cuckoo. *Nature* 397: 667–672.

King, R. A., Symondson, W. O. C., and Thomas, R. J. (2015) Molecular analysis of faecal samples from birds to identify potential crop pests and useful biocontrol agents in natural areas. *Bulletin of Entomological Research* 105(03): 261–272.

Kleven, O., Moksnes, A., Røskaft, E., and Honza, M. (1999) Host species affects the growth rate of cuckoo (*Cuculus canorus*) chicks. *Behavioral Ecology and Sociobiology* 47(1–2): 41–46.

Kleven, O., Moksnes, A., Røskaft, E., Rudolfsen, G., Stokke, B. G., and Honza, M. (2004) Breeding success of common cuckoos *Cuculus canorus* parasitising four sympatric species of *Acrocephalus* warblers. *Journal of Avian Biology* 35(5): 394–398.

Kuznetsova, A., Brockhoff, P., and Christensen, R. (2017) lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* 82(13): 1–26.

Kysucan, M., Samas, P., and Grim, T. (n.d.) Post-fledging interactions between the Common Cuckoo *Cuculus canorus* and its cavity-nesting Common Redstart *Phoenicurus phoenicurus* host.

Lewis, S., Wanless, S., Wright, P. J., Harris, M. P., Bull, J., and Elston, D. A. (2001) Diet and breeding performance of black-legged kittiwakes *Rissa tridactyla* at a North Sea colony. *Marine Ecology Progress Series* 221: 277–284.

Li W. and Godzik, A. (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22(13): 1658–1659.

Martín-Gálvez, D., Soler, M., Soler, J. J., Martín-Vivaldi, M., and Palomino, J. J. (2005) Food acquisition by common cuckoo chicks in rufous bush robin nests and the advantage of eviction behaviour. *Animal Behaviour* 70(6): 1313–1321.

Massimino, D., Woodward, I.D., Hammond, M.J., Harris, S.J., Leech, D.I., Noble, D.G., Walker, R.H., Barimore, C., Dadam, D., Eglington, S.M., Marchant, J.H., Sullivan, M.J.P., Baillie, S.R. and Robinson, R. A. (2017) *BirdTrends 2017: trends in numbers, breeding success and survival for UK breeding birds. Research Report 704.* Thetford.

Mayer, J. (1971) *Ecological relationships of the great reed warbler (Acrocephalus arundinaceus L.) and the reed warbler (Acrocephalus scirpaceus Herm.) during breeding season (in Czech)*. Masaryk University, Brno.

Moksnes, A., and Røskaft, E. (1995) Egg-morphs and host preference in the common cuckoo (*Cuculus canorus*): an analysis of cuckoo and host eggs from European museum collections. *Journal of Zoology* 236(4): 625–648.

Moreby, S. ., and Stoate, C. (2001) Relative abundance of invertebrate taxa in the nestling diet of three farmland passerine species, Dunnock *Prunella modularis*, Whitethroat *Sylvia communis* and Yellowhammer *Emberiza citrinella* in Leicestershire, England. *Agriculture, Ecosystems & Environment* 86(2): 125–134.

Morrison, C. a., Robinson, R. a., Clark, J. a., Risely, K., and Gill, J. a. (2013) Recent population declines in Afro-Palaearctic migratory birds: The influence of breeding and non-breeding seasons. *Diversity and Distributions* 19(8): 1051–1058.

Nakamura, H., and Miyazawa, Y. (1997) Movements, space use and social organisation of radio tracked common cuckoos during the breeding season in Japan. *Japanese Journal of Ornithology* 46: 23–54.

Newton, I. (2004) Population limitation in migrants. *Ibis* 146(2): 197–226.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H. (2019) vegan: Community Ecology Package. R package.

Øien, I., Honza, M., Moksnes, A., and Røskaft, E. (1996) The risk of parasitism in relation to the distance from reed warbler nests to cuckoo perches. *Journal of Animal Ecology* 65(2): 147–153.

Pompanon, F., Deagle, B. E., Symondson, W. O., Brown, D. S., Jarman, S. N., and Taberlet, P. (2012) Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology* 21(8): 1931–1950.

Ratnasingham, S., and Hebert, P. D. N. (2007) BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* 7(3): 355–364.

Rose, L. N. (1982) Breeding ecology of British pipits and their Cuckoo parasite. *Bird Study* 29(1): 27–40.

Sealy, S. G. and Lorenzana, J. C. (1997) "Feeding of nestling and fledgling brood parasites by individuals other than the foster parents: a review." *Canadian Journal of Zoology* 75 (11): 1739-1752.

Seel, D. C., and Walton, K. C. (1979) Numbers of Meadow Pipits *Anthus pratensis* on Mountain farm grassland in North Wales in the breeding season. *The Ibis* 121(2): 147–164.

Sklepowicz, B., and Halupka, L. (2009) The use of sympatric Reed Warblers *Acrocephalus scirpaceus* and Marsh Warblers *Acrocephalus palustris* as breeding hosts: parasitism rates and breeding success of Common Cuckoos *Cuculus canorus*; *Acta Ornithologica* 44(2): 177–184.

Soler, M. (2008) Do hosts of interspecific brood parasites feed parasitic chicks with lower-quality prey? *Animal Behaviour* 76(5): 1761–1763.

Stackebrandt, E., and Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. International Journal of Systematic and Evolutionary *Microbiology* 44(4): 846-849.

Thomson, R. L., Tolvanen, J. and Forsman, J. T. (2016) ‘Cuckoo parasitism in a cavity nesting host: Near absent egg-rejection in a northern redstart population

under heavy apparent (but low effective) brood parasitism', *Journal of Avian Biology* 47(3): 363–370.

Thorup, K., Bulyuk, V., Markovets, M., and Sokolov, L. V. (2018) Kamchatka to Southern Africa: Common cuckoos manage the longest documented land bird migration. In *27th International Ornithological Congress, Vancouver BC Canada August 2018*.

Trevelline, B. K., Nuttle, T., Hoenig, B. D., Brouwer, N. L., Porter, B. A., and Latta, S. C. (2018) DNA metabarcoding of nestling feces reveals provisioning of aquatic prey and resource partitioning among Neotropical migratory songbirds in a riparian habitat. *Oecologia* 187(1): 85–98.

Trnka, A., Požgayová, M., Procházka, P., Prokop, P., and Honza, M. (2012) Breeding success of a brood parasite is associated with social mating status of its host. *Behavioral Ecology and Sociobiology* 66(8): 1187–1194.

Tyller, Z., Kysučan, M., and Grim, T. (2018) Postfledging behavior of the Common Cuckoo (*Cuculus canorus*) attended by the Chaffinch (*Fringilla coelebs*): a comprehensive approach to study the least-known stage of brood parasite–host coevolution. *The Wilson Journal of Ornithology* 130(2): 536–542.

van Klink, R., Mandema, F. S., Bakker, J. P., and Tinbergen, J. M. (2014) Foraging site choice and diet selection of Meadow Pipits *Anthus pratensis* breeding on grazed salt marshes. *Bird Study* 61(1): 101–110.

Vandenberghe, C., Prior, G., Littlewood, N. a., Brooker, R., and Pakeman, R. (2009) Influence of livestock grazing on meadow pipit foraging behaviour in upland grassland. *Basic and Applied Ecology* 10(7): 662–670.

Vega, M. L., Willemoes, M., Thomson, R. L., Tolvanen, J., Rutila, J., Samaš, P., Strandberg, R., Grim, T., Fossøy, F., Stokke, B. G., and Thorup, K. (2016) First-Time Migration in Juvenile Common Cuckoos Documented by Satellite Tracking. *PLOS ONE* 11(12): e0168940.

Walton, K. C. (1979) Diet of meadow pipits *Anthus pratensis* on mountain grassland in Snowdonia. *Ibis* 121(3): 325–329.

Willemoes, M., Strandberg, R., Klaassen, R. H. G., Tøttrup, A. P., Vardanis, Y., Howey, P. W., Thorup, K., Wikelski, M., and Alerstam, T. (2014) Narrow-front loop migration in a population of the common cuckoo *Cuculus canorus*, as revealed by satellite telemetry. *PloS one* 9(1): e83515.

Wilson, J. D., Arroyo, B. E., and Clark, S. C. (1996) *The diet of bird species of lowland farmland: a literature review. Unpublished report to the Department of the Environment and Joint Nature Conservation Committee*. Sandy, Bedfordshire.

Wyllie, I. (1981) *The Cuckoo*. London: Batsford.

Ying H., Beifang N., Ying G., Limin F., and Weizhong L. (2010) CD-HIT Suite: a web server for clustering and comparing biological sequences. *Bioinformatics* 26(5): 680–682.

Zeale, M. R. K., Butlin, R. K., Barker, G. L. A., Lees, D. C., and Jones, G. (2011) Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources* 11(2): 236–244.

5. Assessing breeding ground diet of the common cuckoo *Cuculus canorus* using two novel methods

Abstract

The habitat-specific patterns of decline in common cuckoo *Cuculus canorus* breeding abundance in Britain reflect patterns in adult population change of moths whose larvae are recorded as cuckoo prey. Declines are pronounced in woodland, arable land and improved grassland compared to stability in semi-natural habitats. Prey availability is also a potential driver of cuckoos' non-breeding season movements, and there is initial evidence the cuckoo relies on breeding ground prey supply to enter breeding condition after arrival. However, the diet of the cuckoo has not been studied in detail since before the period of most significant decline in Britain, which began in the mid 1980s. Regarded as a specialist of large moth and butterfly (Lepidoptera) larvae, there is little direct evidence that the cuckoo is reliant on this taxon. The present study included a localized molecular ecology analysis of the diet of adult cuckoos in a relative stronghold upland habitat in England, and a remote 'observational' study of cuckoo prey across Britain through digital photographs of adult and juvenile prey-handlings across 14 breeding seasons 2003-16, uploaded to social media libraries. The molecular study amplified and sequenced invertebrate DNA from faecal samples collected from cuckoos during ringing and freshly deposited beside natural perches on Dartmoor. Analysis of the occurrence of DNA from arthropod taxa in faeces suggested high frequency of Lepidoptera, including Lasiocampidae moths widely reported as prey by other methods; but also high frequency of grasshoppers (Orthoptera) and flies (Diptera) likely to be overlooked by observational studies. Sequencing also revealed possible alternative non-Lepidopteran prey taken in cooler springs. Analysis of photographs largely concurred with past observational studies regarding the characteristics of Lepidoptera larvae taken as prey by the cuckoo. Adults throughout Britain were most frequently captured predating large Lasiocampidae larvae while juveniles most frequently predated cinnabar moths *Tyria jacobaeae*. The most frequent prey included taxa widely reported to be vulnerable to intensive changes in land-use practice. The study findings suggest

that historical cuckoo range contraction in lowlands related to reduction in invertebrate food supply, and that restoring cuckoo populations requires extensive application of low intensity land use methods.

Introduction

Reliance by a predator on a narrow range of prey species is expected to expose it to greater impact of environmental changes that deplete prey populations. The common cuckoo *Cuculus canorus* is a migratory brood-parasitic bird that has declined in many parts of its Palaearctic breeding range, and is thought to feed predominantly on large larvae (caterpillars) of butterfly and moth species (Lepidoptera). Monitoring suggests there have been significant declines in adult abundance of many Lepidoptera species in Britain (Conrad *et al.* 2006). For species recorded as cuckoo prey (Wyllie 1981), their overall abundance change (as recorded by the Rothamsted Insect Survey) shows strong correlation with land use, with significant percentage declines in woodland, improved grassland and arable land, compared to significant percentage increases in semi-natural habitats (Denerley *et al.* 2018). These habitat specific trends strongly reflect those for cuckoo breeding abundance (Massimino *et al.* 2017). Prey abundance is therefore a potential driver of cuckoo population change in Britain. However, the adult diet of the cuckoo is not well understood, and has not been studied in detail in any population for decades. Apparent specialisation on large caterpillars as prey may be an artefact of the field observation-based and often anecdotal approach to prey studies in the historic literature on cuckoo prey and feeding (Wyllie 1981). Larger larvae with physical defences (hairs and spines) require more handling before ingestion, and may carry more distinct features that allow finer identification at a distance. Similarity of movement of cuckoos to that of other large insectivorous migrant birds (Jacobsen *et al.* 2017) and with seasonal changes in vegetation 'greenness' and associated herbivorous insect abundance (Thorup *et al.* 2017), suggest non-breeding movements also closely track prey availability. Early prey availability in the breeding area appears to be vital for adult birds reaching breeding condition after arrival from western Africa (Wyllie 1981). While cuckoos mainly arrive in Britain in late April, the (first) peak

egg laying date as analysed in meadow pipit *Anthus pratensis* nests (the main host in our study area) was in mid May (Rose 1982). Cuckoos are also anecdotally observed to spend much of their time feeding early in their spring season in Britain (Condry 1966, Wyllie 1981). These are preliminary indications that cuckoos are 'income breeders', which feed on food resources present on the breeding grounds to restore and maintain their body condition over the breeding period, compared to 'capital' breeders which use the body condition resultant from feeding earlier in the annual cycle to sustain them through the breeding period (Drent & Daan 1980). Identification of prey taken, in its full diversity and over the full duration of the cuckoo breeding season (and ultimately the annual cycle), is essential to refine our understanding of how cuckoo populations may be driven by prey abundance. In the present study I used novel methods for identifying prey at a local and national scale in Britain. At the local scale I used molecular analysis of DNA in cuckoo faeces to identify prey of all taxa and size classes taken by cuckoos in a relative stronghold area of semi-natural upland grassland in south-west England. At the national scale, I identified species of Lepidoptera taken as prey by cuckoos in the period 2003-2016 as documented in digital photographs uploaded to social media libraries.

Study of cuckoo prey is challenging, chiefly because of the large individual home ranges, low population density, and the short season during which adults are on the breeding grounds, which has been measured for northern European breeders as averaging just 49 days (Willemoes *et al.* 2014). Individual tracking studies have consistently demonstrated that cuckoos commute to feed in locations entirely separate from their breeding area both in terms of distance and habitat type and individuals can range over several square kilometres (Wyllie 1981, Droscher 1988, Nakamura & Miyazawa 1997, Vogl *et al.* 2004). Chance (1940) and Davis (1996) both noted cuckoos disappearing from study areas for long periods, consistent with commuting to feed as documented in tracking studies. While birds foraging in open habitat may be directly observable, some tracking studies indicate foraging in enclosed habitats such as scrub and woodland where feeding and prey are not observable. In all habitats a low population density limits the rate of encounter with different individuals, and the distance of observation is likely to bias prey identification

towards the largest prey. Most studies of cuckoo diet pre-date the period of significant decline in breeding abundance (post-1985, BTO (British Trust for Ornithology) Common Birds Census CBC/Breeding Birds Survey BBS, Massimino *et al.* 2017). Prey of juveniles before first migration from Europe is known from limited observational studies to resemble prey of adult cuckoos – large caterpillars (Wyllie 1981). The extent to which cuckoo juveniles remain and forage for themselves in the natal area before migrating is unknown.

Across studies of cuckoo diet to date, prey has comprised predominantly invertebrate animals. All field observation studies have cited larvae of moths and butterflies (Lepidoptera) as the main prey taxon (Condry 1966, Bottomley & Bottomley 1975, Armitage 1978). This has support from stomach contents studies, although in some cases specimens have contained almost entirely beetle (Coleoptera) remains (Collinge 1925, Lowe 1943, Dement'ev & Gladkov 1966, Ishazawa & Chiba 1966). Of these, Lowe (1943) refers to just four specimens, while two studies examined around 20 stomachs (Collinge 1925, Dement'ev & Gladkov 1966), and Ishazawa & Chiba (1966) systematically analysed 82 stomachs. An over-arching observation from past studies is intensive consumption of a single taxon of high visibility and abundance. This has led some authors to conclude that cuckoo foraging is less a specialist on Lepidoptera larvae and more a flexible consumer of the most abundant and visible invertebrate taxa (Lowe 1943). However, stomach contents studies, by definition, present 'snapshot' observations of animal diet. With the exception of invasive stomach-flushing studies of live birds, individuals are deceased and contents relate only to their final one or few food intakes. Reduced opportunity to record individual variation, due to methodology, is more likely to (artificially) indicate low individual prey diversity, or specialism. Individual diet would be captured over longer periods by observation of, or faecal sampling from, individuals.

On current evidence Lepidoptera is the most common prey taxon. Many species in this order are known to have declined significantly in the UK (Conrad *et al.* 2006). There is also indication of a long-term decline in flying insect biomass in Western Europe (Hallmann *et al.* 2017). The lepidopteran species known to be predated by cuckoos are mainly moth species with large larvae, armed with

physical or chemical defences that repel many bird species from consuming them (Wyllie 1981). Study of the bill and jaw anatomy of common cuckoos and other Cuculidae shows adaptations that increase ability to handle prey at the bill base, and disarm toxic prey or subdue larger prey (Korzun *et al.* 2003). Cuckoos are able to shed their stomach lining (McAtee 1906) which *in vivo* has been sometimes found lined with irritant caterpillar hairs (McAtee 1917). These characteristics suggest that cuckoos have adapted to feed on large defensive invertebrates in high volume. Moths whose caterpillars are prey species of the cuckoo, as collated by Wyllie (1981), are recorded to have undergone greater population declines than the average across moth species in the UK (Denerley *et al.* 2018). That these species' populations show similar inter-habitat differences in population trend (Denerley *et al.* 2018) to those for the cuckoo (Massimino *et al.* 2017), is an indication that changing abundance of suitable invertebrate prey may play a role in cuckoo population decline.

In the previous chapter, the prey of cuckoo nestlings and fledglings in upland semi-natural grassland in England was found to feature different frequencies of several invertebrate taxa compared to prey of host nestlings. Of these, Diptera were found in significantly greater frequency in cuckoo faecal samples, and dipteran prey detected were of a range of body sizes from Chloropidae (grass flies) to Rhagionidae (snipeflies). Fledgling cuckoos were provisioned at a greater rate than nestling cuckoos or hosts. It was concluded that nestling cuckoos may benefit from a diverse availability of invertebrates, as host pairs raising cuckoos showed reduced or shifted prey selection compared to those raising host broods. Diversity and abundance of invertebrates in this relative stronghold habitat for cuckoo may explain lack of cuckoo parasitism in agriculturally intensive lowland habitats where previously common hosts still thrive. In the present study, I used DNA sequencing methods to identify prey taxa from faecal samples of adult cuckoos, collected in upland semi-natural breeding habitat in England. Prey analysis focused on amplifying, segregating and reading sequences of a 157-212 base pair (bp) length region of the cytochrome-b oxidase I (COI) gene found in animal mitochondrial DNA (mtDNA). Sequencing of DNA extracted from organisms of known identity shows this region to be significantly conserved within, and variable between,

animal taxa, commonly at the level of species. The gene region can therefore provide a 'barcode' DNA sequence. The aim was to identify cuckoo prey in a habitat where the breeding population is stable or increasing compared to other UK habitats (Massimino *et al.* 2017). DNA-amplification (PCR) based study of prey in predator faeces (reviewed King *et al.* 2008) is likely to carry less detection bias for large prey than field observation; is non-invasive compared to stomach contents studies; and presents an alternative to the low-resolution and labour-intensive option of identifying prey from microscopy of faeces (Pompanon *et al.* 2012), which may also fail to detect remains of soft-bodied prey (Barrett *et al.* 2007).

Secondly, I used high resolution photographs accessible on photographic social media platforms such as Flickr, to collate observations of cuckoo prey species in the UK over the last two decades. Crowd-sourced photography across an effective study area of national scale has been a successful recent tool for studying diet across seabird colonies (RSPB 2017). Crowd-sourcing of data including photographs via social media platforms has received recent attention as a remote-sensing tool in ecology and conservation science (Richards and Friess 2015, Di Minin *et al.* 2015, Jeawak *et al.* 2017).

Method

1. Diet in a stronghold habitat area from molecular analysis of faeces

Study sites and field sample collection

We collected faeces from common cuckoo adults present between April and June 2017 (and two birds ringed in May 2016) in Dartmoor National Park, UK (Fig. 1 inset). Cuckoo faecal samples were primarily obtained in the field by collecting faeces dropped by birds using natural perches (trees, shrubs and rocks). Further samples were collected through supplementary mist-netting and ringing effort. Warren House (50° 36' 43" N, 03° 52' 13" W) and Burrator (50° 31' 24" N, 04° 0' 55" W) were study areas selected for their suitable topography for finding and maintaining visual track of adult cuckoos, and for approaching and sampling from areas underneath perches. Both sites comprised valleys with

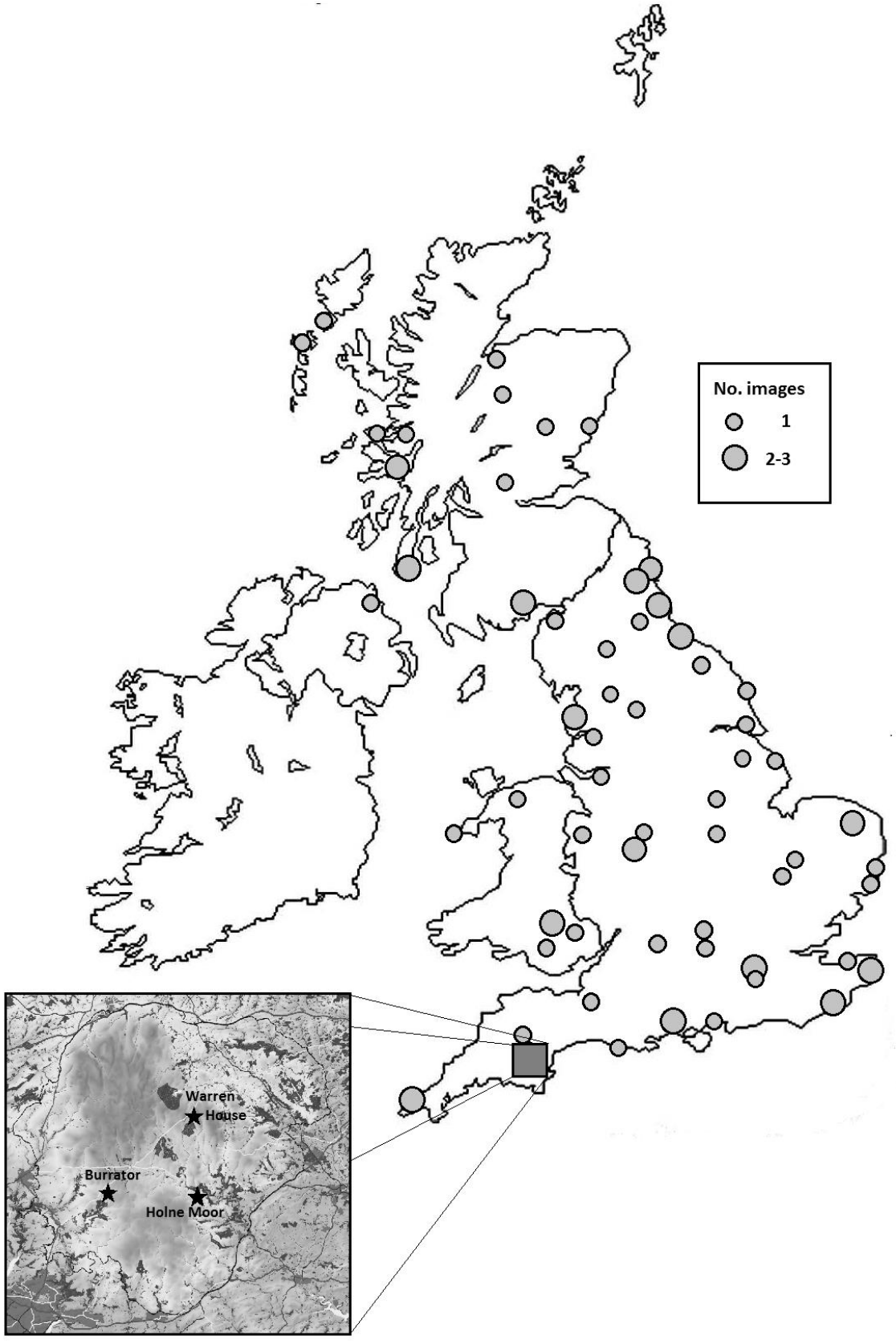


Figure 1. UK locations of cuckoos handling prey in images included in analysis, and faecal sampling locations in Dartmoor National Park (inset)

scattered trees on both sides, surrounded by moorland generally lacking trees. Most perches were well separated and under 4 m tall and there was relatively short vegetation beneath perches due to grazing. Sites were walked in a route with generally high visibility over the study area from 06:00, listening and scanning visually for cuckoos. This was carried out in most weather and visibility conditions except for combined wet and windy conditions. Once located, cuckoos were watched and followed continuously until defecation took place. Faeces were searched for within 20 minutes, allowing time to watch for further dropping of faeces or to note the bird's movement and new location if it vacated the perch. Search areas were approached in a straight line from the vantage point, maintaining focus on the perch location of the bird until directly underneath. All visibly fresh faeces that were located were collected into separate 5 ml vials containing 80-90% ethanol. Vials were refrigerated at +5 °C within 12 h (but see Discussion).

Mist-netting was carried out at Holne Moor (50° 31' 20" N, 03° 51' 43" W), where cuckoo density was similar to Warren House and Burrator but topography was flatter and suitable habitat more extensive, hindering efficient tracking and sampling as above. Trapping took place under BTO Ringing Scheme license with mist-net endorsement. Effort ran from 04:00 until mid morning when cuckoo activity dropped. Following a combination of methods from Vogl *et al.* (2004) and Noakes (2013), a triangle orientation of three 18 m mist-nets (30 x 30 mm mesh) (Ecotone, Sopot, Poland) was used, with a decoy female cuckoo at the centre and a sound lure of male and female cuckoo breeding calls, at volume recommended by BTO guidelines (Blackburn *et al.* 2006). Trapped birds were processed through normal ringing and measurement for the BTO Ringing Scheme and before release were placed individually for ten minutes in a blacked out fabric veterinary carrycase with a clean laminated card floor for each new bird. All faecal material released over the ten minutes was collected into a single 5 ml vial containing 80-90% ethanol and kept at 5° C within 12 h (but see Discussion).

DNA extraction and sequencing

Prior to extraction, samples were freeze-dried to remove ethanol from vials and subsequently held at -80 °C. DNA was extracted using a precipitation and re-suspension method (Chaput unpublished 2017). To confirm the origin bird species of each faecal sample as common cuckoo, extracted DNA from each sample was used as template DNA in PCR (polymerase chain reaction) with the primer pair AvMiF1 and BirdR1 (Hebert *et al.* 2004, Kerr *et al.* 2009), adopted for targeting a short region (~290bp) of the avian COI gene in faeces. As discussed above for invertebrate identification, this gene is conserved within bird taxa including at the species level, allowing identification of species matches from established databases of known species-level 'barcode' sequences. PCR products were visualised on agarose gels to ensure amplification of expected sequence length, and were then purified using QIAquick gel extraction kit (Qiagen, Hilden, Germany). Purified PCR products were Sanger sequenced externally against AvMiF1 primer (MWG Eurofins, eurofinsgenomics.eu). Returned sequences were entered as queries to the Barcode of Life Database (BOLD) version 4 (Ratnasingham and Hebert 2007), searching species-level barcodes. Samples were identified as common cuckoo if their tested avian COI sequence matched a sequence from a species of genus *Cuculus* with ≥95% similarity.

DNA from cuckoo samples was amplicon-sequenced using the Illumina MISEQ system. Libraries for amplicon sequencing were prepared by amplifying a region of COI mtDNA from each sample's extracted DNA via PCR (polymerase chain reaction), using the general invertebrate primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al.* 2011). For each sample entering PCR, primers carrying a different unique 5' adaptor sequence were used, to allow identification of sample origin for all sequences read during the MISEQ run. For further details of molecular methods see chapter 2.

Data analysis

DNA sequence reads from Illumina MISEQ runs were de-multiplexed according to the 5' adaptor used in PCR of DNA from each faecal sample. In the R package Dada2 (Callahan *et al.* 2016), sequences were clipped of the first 30

bp and 24 bp from the forward and reverse reads of sequences, respectively, to remove primer sequences. Reads were truncated at 200 bp and truncated where base call accuracy fell below Q35. Reads were dereplicated in Dada2 which was used to identify amplicon sequence variants (ASVs). Each ASV was entered as a query to a search of all species-level barcodes (3,181,157 sequences, 192,424 species, as of July 2018) in the Barcode of Life Database (BOLD) version 4 (Ratnasingham & Hebert 2007). Species barcode sequences were considered matches if similarity exceeded 97% (following Stackebrandt & Goebel 1994). The species with the highest similarity above this threshold was recorded following King *et al.* (2015). Species identifications for each unique sequence, and the total number of reads in each faecal sample were exported into a summarising table. All ASV sequences from MISEQ with similarity of database sequences greater than 98% (following Clare *et al.* 2011 and King *et al.* 2015) were retained. Sequences matching prokaryotic taxa were excluded. Species names linked to each top matching sequence were searched online. For all sequences for which the species had no records of UK occurrence, the taxon reported here was the next taxonomic grouping upwards from that species for which there are UK records, e.g. same genus, family. I clustered samples collected fresh from the same ~3 x 3 m area at the same time, as these samples were strongly likely to originate from the same bird. These clusters are in subsequent text referred to as 'sampling events'. I calculated frequency of invertebrate orders, families and species against two different denominators; firstly, as a percentage of all faecal samples in which a sequence matching the taxon was detected, and secondly, as a percentage of sampling events from which a sequence matching the taxon was detected. Lepidoptera identifiable to species level with UK records were visualised by sampling month for direct comparison with monthly occurrence of larvae detected nationally from photographs (see below). For each species, the life stages (adult or larvae) reported in the literature to be available in southern Britain during the sample month were used to determine which life stage of the species was likely to be consumed by cuckoos given the date DNA was detected. Adult and larval months of macro moth taxa were those given by Waring and Townsend (2017). Those for micromoth taxa were those given by UKmoths.org.uk (2018).

2. Identifying cuckoo prey in Britain from digital photographs

Sampling method

Images were sourced by individually surveying the thumbnail results of keyword searches of web based image libraries. The search terms “cuckoo” and “Cuculus”. were entered in turn into the search tools of Flickr (www.flickr.com), Birdforum Gallery (http://www.birdforum.net/gallery/search .php), Birdguides Gallery (formerly Iris, http:// www.birdguides.com/gallery/) and Google Images (http://images.google.com). For incorporation into the data set, images met the following criteria. i) Image contained common cuckoo *C. canorus* handling a prey item, ii) the cuckoo was not a nestling or fledgling being provisioned by a host foster parent, iii) the image was a photograph of sufficient resolution to attempt identification of the prey item, iv) the image and accompanying information gave no suggestion that the prey or food was artificially provided, v) the image information identified (at minimum) the country and month in which it was taken. While images captured in Britain were the priority of this study, as a country which has seen overall long term cuckoo decline with regional variation in trend (Harris *et al.* 2018), we retained images of cuckoos handling prey from across the global range. The plumage of the cuckoo in each image was used to age the bird as juvenile or adult. Prey items in images were identified to lowest possible taxonomic level, with all identifications made by the same person (B. Henwood) with expertise in larvae of British Lepidoptera and other invertebrates. Each identification was also ascribed a qualifying value regarding percentage certainty on the identification made.

Data analysis

Images where the identity of the prey was ascribed 80% certainty or higher by the taxonomic expert (B. Henwood) were included in subsequent analysis. The geographic distribution of captured images extended to the far north and south of Britain, with photographs originating from inland and coastal sites, though photographs in Scotland were more clustered to coastal and island locations and relatively few photographs were available from Wales (Fig. 1). The appearance, date, location and accompanying information were used to identify images of the same bird and prey item (referred to in subsequent text as prey

handling events). This was the unit used in calculating total sample size and number of images of each identified prey.

Results

1. Diet in a stronghold habitat area from molecular analysis of faeces

Of 59 faecal samples collected across the two field sampling techniques (7 from trapping and ringing, 52 from field sample collection), 48 were confirmed to be common cuckoo by Sanger sequencing of avian COI mtDNA (7 from trapping and ringing and 41 from field sample collection). MISEQ runs were completed for 41 faecal samples which clustered to 34 sampling events. 170 unique sequences matched eukaryote taxa with similarity ≥98 %, and these sequences accounted for 2169857 reads on the MISEQ platform. Sequences matched to arthropods of two classes (Insecta and Arachnida) and seven orders (Lepidoptera, Orthoptera, Diptera, Coleoptera, Hemiptera, Plecoptera,

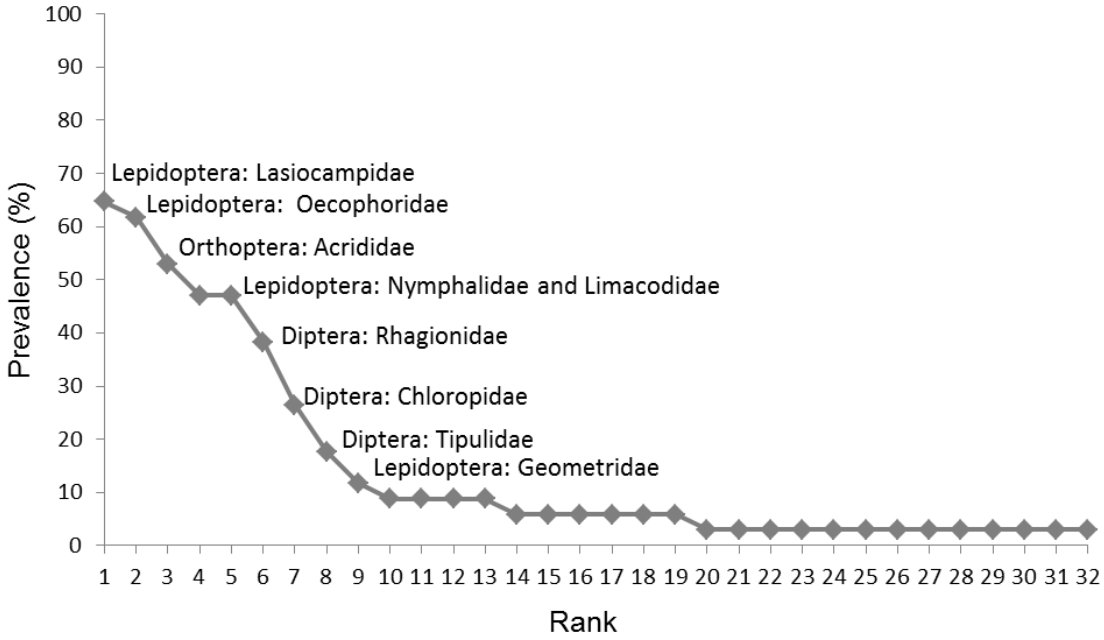


Figure 2. Rank-prevalence curve of arthropod families in common cuckoo diet in Dartmoor, UK study area according to COI DNA sequences detected in faeces 'sampling events' (n=34) following PCR with ZBJ-Art primers (Zeale *et al.* 2011) (Table 1).

Table 1. Frequency of occurrence of eukaryote taxa matched with ≥98% to DNA sequences, in faecal samples (and clusters of co-occurrent samples, 'sampling events') from common cuckoo adults in Dartmoor National Park, UK in 2016-17 breeding seasons, as detected by Illumina MISEQ amplicon sequencing. 'Unknown' species denote species-level matches with no previous UK records, here treated as unidentified species in the same higher taxon e.g. genus or family. 'Total' frequencies include frequency of constituent sequences from both UK-occurring species, and 'unknown' species as above. WH = Warren House, BU = Burrator, HM = Holne Moor.

Class	**Order**	**Family**	Species	% frequency (samples, n = 41)	% frequency ('sampling events', n=34)	Sites
Arachnida	**Sarcoptiformes**	**[Total]**		**12.2**	**14.7**	
		Chamobatidae	*Chamobates pusillus*	7.3	8.8	WH, BU
		Crotoniidae	*Platynothrus peltifer*	4.9	5.9	BU, HM
		Punctoribatidae	*Punctoribates punctum*	2.4	2.9	BU
Insecta	**Coleoptera**	**[Total]**		**12.2**	**14.7**	
		Dascillidae	*Dascillus cervinus*	2.4	2.9	WH
		Scarabaeidae	*Phyllopertha horticola*	4.9	5.9	WH, BU
		Staphylinidae	*Ocypus aeneocephalus*	7.3	8.8	WH, BU
Insecta	**Diptera**	**[Total]**		**48.8**	**52.9**	
		Anthomyiidae	*Hylemya variata*	2.4	2.9	BU
		Bibionidae	*Dilophus febrilis*	2.4	2.9	WH
		Chloropidae	(total)	24.4	26.5	WH, BU, HM
			Tricimba sp.	14.6	14.7	WH, BU, HM
		Culicidae	(total)	7.3	8.8	BU
		Limoniidae	*Limonia nubeculosa*	2.4	2.9	BU
		Pediciidae	*Tricyphona immaculata*	7.3	8.8	WH, BU
		Psychodidae	*Psychoda trinodulosa*	2.4	2.9	HM

		Rhagionidae	(total)	34.1	38.2	WH, BU, HM
			Rhagio scolopaceus	14.6	17.6	WH, BU, HM
			Rhagio tringarius	22.0	23.5	WH, BU, HM
		Tipulidae	(total)	14.6	17.6	BU, HM
			Tipula luna	2.4	2.9	BU
			Tipula paludosa	14.6	17.6	BU, HM
Insecta	**Hemiptera**	**[Total]**		**4.9**	**5.9**	
		Miridae	*Lygocoris rugicollis*	2.4	2.9	BU
		Psyllidae	*Cacopsylla melanoneura*	2.4	2.9	BU
Insecta	**Lepidoptera**	**[Total]**		**75.6**	**73.5**	
		Erebidae	(unknown)	4.9	5.9	WH, BU
		Geometridae	(total)	14.6	11.8	WH, BU
			Petrophora chlorosata	7.3	8.8	WH, BU
			Pseudoterpna sp.	2.4	2.9	BU
		Glyphipterigidae	*Glyphipterix fuscoviridella*	4.9	5.9	WH, BU
		Lasiocampidae	(total)	65.9	64.7	WH, BU
			Euthrix potatoria	51.2	47.1	WH, BU
		Limacodidae	(unknown)	43.9	47.1	WH, BU
		Lycaenidae	(unknown)	4.9	5.9	WH, BU
		Noctuidae	*Xestia agathina*	7.3	5.9	WH
		Nymphalidae	(unknown)	46.3	47.1	WH, BU
		Oecophoridae	(unknown)	58.5	61.8	WH, BU, HM
		Saturniidae	*Saturnia pavonia*	2.4	2.9	BU
		Thyrididae	(unknown)	2.4	2.9	WH
		Tortricidae	*Crocidosema plebejana*	2.4	2.9	BU
		Ypsolophidae	*Ochsenheimeria urella*	2.4	2.9	WH, BU

Insecta	**Orthoptera**	Acrididae	*Omocestus viridulus*	**51.2**	**52.9**	WH, BU, HM
Insecta	**Plecoptera**	Leuctridae	*Leuctra hippopus*	**2.4**	**2.9**	BU
Bdelloidea	Incertae_sedis	Philodinidae	*Macrotrachela quadricornifera*	2.4	2.9	BU
Eutardigrada	**Parachaela**			**7.3**	**8.8**	
		Hypsibiidae	*Isohypsibius sattleri*	4.9	5.9	WH, BU
		Macrobiotidae	*Murrayon dianeae*	2.4	2.9	BU

Sarcoptiformes), plus one class and order each of Rotifera and Tardigrada which were considered to be environmental sources of contaminant DNA as opposed to genuine cuckoo prey. The most frequent order sequenced from faeces was Lepidoptera, found in 73.5% of sampling events. DNA of Diptera and Orthoptera were both detected in more than half of sampling events (52.9% in both cases, Table 1). Diptera families Rhagionidae (snipeflies), and Chloropidae (grass flies) were detected in more than 20% of sampling events (Fig. 2). All Orthoptera sequence matches referred to the family Acrididae and the species *Omocestus viridulus* (common green grasshopper). The most frequently detected Lepidoptera family was Lasiocampidae (64.7% of sampling events), predominantly *Euthrix potatoria* (drinker moth), followed by micromoth family Oecophoridae (concealer moths, 61.8%) (Fig. 2), though species-level identifications did not include UK-occurring genera so identification was restricted to family level. Considering all UK Lepidoptera species sequenced against sample months, *E. potatoria* was detected in all months (April to June), and additionally in April faecal samples contained DNA of *Xestia agathina* and micromoth *Ochsenheimeria urella* (Fig. 3). Non-Lepidoptera DNA present in samples collected in April (n = 4 sampling events sequenced) referred to Orthoptera (*O. viridulus*), Coleoptera (*Ocypus aeneocephalus*), Diptera (*Rhagio tringarius*) and Sarcoptiformes (*Chamobates pusillus*). Most Lepidopteran species were sequenced from samples collected in May (Fig. 3) but this was also the month where collection of samples peaked (n = 26 sampling events sequenced).

2. Britain and Ireland prey ID from digital photographs

Photographs of 42 prey handling events in adult cuckoos, and 22 in juveniles, met all study criteria and contained prey identified with sufficient certainty for inclusion in analysis. Adult cuckoos were recorded predating *E. potatoria* and *Lasiocampa quercus* (oak eggar) in England, Wales and Scotland, with one or both of these lasiocampids detected in all months where photographs were available of adults (Fig. 3a). Images of these taxa accounted for 38 prey events,

a)	April	May	June (plus 1st July)
England (n = 22 prey event images)	*Euthrix potatoria* (13)  *Lasiocampa quercus* (7)  *Euproctis chrysorrhoea* (1)  unidentified Noctuidae (1) 		
Dartmoor National Park (molecular study, n = 34 sample clusters)	*Euthrix potatoria* (16) (larvae) (+ 6 unidentified Lasiocampidae)  *Pseudoterpna sp.*(1) (larvae)  *Petrophora chlorosata* (3) (adult)  *Xestia agathina* (2) (larvae)  *Saturnia pavonia* (1) (larvae, poss. adults)  *Ochsenheimeria urella* (1) (larvae)  *Glyphipterix fuscoviridella* (2) (adults/larvae)  *Crocidosema plebejana* (1) (larvae) 		
Wales (n=6 prey event images)	*Euthrix potatoria* (4)  *Lasiocampa quercus* (2) 		

Scotland (n = 14 prey event images)	*Euthrix potatoria* (9) <----------> *Lasiocampa quercus* (3) <----------> *Arctia caja* (2) <----------<---------->

b)	July	August	September	October
England (n = 20 prey event images)	*Tyria jacobaeae* (13) <----------> *Zygaena filipendulae* adult (1) <----------> *Aglais* or *Vanessa sp.* Nymphalidae (2) <----------> *Pieris brassicae* (2) <----------> *Macrothylacia rubi* (2) <---------->			
Wales (n = 1)	*Zygaena filipendulae* adult (1) <---------- <---------- [No data]---------->			
Scotland (n = 1)	*Tyria jacobaeae* (1) <----------> <----------[No data]---------->			

Figure 3. Occurrence by month of Lepidoptera prey of a) adult and b) juvenile common cuckoos in UK study areas, in photographic images 2003-2016 (England, Wales, Scotland) and sequencing of prey DNA from faeces (Dartmoor in England). All prey in images were larvae unless stated otherwise. In Dartmoor section, 'larvae' and 'adults' denotes life stage(s) are cited to be available as prey during the period when DNA was detected, indicating whether larvae or adults were predated.

90.5% of the total, and the remainder of images captured three further Lepidopteran prey taxa. *Arctia caja* (garden tiger) was captured in images from Scotland only, while the larger sample of images from England contained *Euproctis chrysorrhoea* (brown tail moth) and unidentified Noctuidae moths (Fig. 3a). Very few images of juvenile cuckoos with prey were available from Scotland or Wales (total n = 2 prey events included in analysis), all captured in July, while in England images were available for July to October (Fig. 3b). The most frequent and temporally dispersed Lepidopteran prey was *Tyria jacobaeae* (cinnabar), but some juveniles remained in England later than the documented larval period of this species ends (early September, Waring & Townsend 2017). Images from September and October showed juveniles feeding on *Pieris brassicae* (large white butterfly) and *Macrothylacia rubi* (fox moth) larvae. As well as larvae, juvenile cuckoos were also captured predating adults of *Zygaena filipendulae* (six-spot burnet) from vegetation.

Discussion

Most samples collected from common cuckoo adults were obtained by tracking and sampling from free-flying birds at suitable sites (see Methods) compared to numbers obtained through trapping. Sanger sequencing of avian DNA from faeces allowed confirmation of faecal sample origin post hoc. This study has demonstrated that field-based collection from free-flying birds of open habitat, combined with molecular biology techniques, can successfully collect significant numbers of faecal samples from species that are difficult to trap such as the common cuckoo (Noakes 2013). In contrast to trapping difficulty, cuckoos readily perched in highly visible locations and defecated at as little as 10 min intervals. Disadvantages of this technique are that independence of samples is not known with certainty, in the absence of individual marking of birds. Contamination from the substrate before collection may take place although contamination with invertebrate or bird DNA is less likely than that from prokaryotes or other microorganisms. In addition to the advantage in sample size, the methodology ensured that faeces were collected while fresh, and sampling carried lower potential impact to the welfare of individuals than

trapping and handling. Faecal sampling from bats, and confirmation of bat species, has been similarly carried out non-invasively on the ground beneath roosts (Bohmann *et al.* 2011). Efficiency of collection of pellets from African marsh harriers *Circus ranivorus* was increased by provision of artificial perches (Simmons *et al.* 1991).

This study is the first to examine prey of the common cuckoo by molecular biology methods, which have enabled detection of hard and soft-bodied organisms of sizes ranging by three orders of magnitude (Table 1). Among the smallest organisms, DNA of tardigrades and rotifera was concluded to have been present within consumed prey or in the environment at point of prey ingestion or faecal egestion. Sarcoptiform mites are non-parasitic soil arthropods of body length ~0.5 mm. DNA of these mites may similarly have originated in the prey of species consumed by the cuckoo, or have been present in the environment at ingestion or egestion. The most frequent, likely genuine prey order was Lepidoptera and the most frequent family was a taxon previously reported widely in common cuckoo prey, Lasiocampidae (Table 1). Drinker *E. potatoria* was found in almost half of sampling events, and was also identified as prey from observation of cuckoos feeding during field faecal sampling efforts. Unidentified lasiocampid DNA is most likely to represent either this species or oak eggar *L. quercus*. These two species appear to be the most common and widespread lasiocampids on Dartmoor. DNA of oecophorid moth origin was also frequent among faecal samples. There are 27 species cited as occurring in the British Isles (UKmoths.org.uk 2018), of which perhaps the most likely to be common on Dartmoor based on range and larval foodplant are *Batia lambdella* or *Pleurota bicostella*. However, several other Oecophoridae which are generalist feeders on dead wood as larvae could account for the DNA detected. These are relatively tiny species of Lepidoptera, and the fact that some invertebrates detected in cuckoo faeces such as Rhagionidae are predatory means it was possible DNA of small invertebrate species were introduced as contaminants following their predation by cuckoo prey. DNA sequences representing nymphalid butterflies were frequent among samples but could not be identified to species level. Dartmoor has abundant populations of several species in this family including small heath *Coenonympha pamphilus*

and generalist widespread species such as gatekeeper *Pyronia tithonus* and small tortoiseshell *Aglais urticae,* therefore inferring prey species is less straightforward. The common green grasshopper *O. viridulus* was detected in more than half of sampling events, and is reportedly the most common orthopteran on Dartmoor (Davies 1987). This species has the earliest spring phenology of British orthopterans, with nymphs emerging in April and moulting to adult (imago) in June (Benton 2012). Orthoptera were previously reported as cuckoo prey (Abbey 1909, Ishizawa & Chiba 1966, Wyllie 1981) but the high frequency with which they occurred in this study was unexpected. In the only quantitative study amongst the above, Orthoptera were found only in one stomach specimen of 50 examined (Ishizawa & Chiba 1966). However, Orthoptera is a frequent prey taxon of Nearctic species of Cuculidae cuckoos during the late breeding season (Beal 1898). Diptera families of greatest frequency were generally of large body-size and spend prolonged periods at rest on grass and other herb foliage. Such prey are likely to suit cuckoos' visual foraging strategy.

A key aim of the present study was analysing prey of the cuckoo in the earliest part of the breeding season, where spring temperatures can significantly affect emergence and growth of overwintering Lepidoptera larvae such as the Lasiocampidae (Wyllie 1981) (Fig. 3a). Early prey availability in the breeding area (as opposed to prey availability at staging areas on migration to the breeding area) appears to be vital for adult birds reaching breeding condition after arrival from western Africa (Wyllie 1981). Cuckoos spend considerable time feeding during their first weeks in the breeding region, and egg laying is uncommon during this period (Lack 1968, Wyllie 1981), suggesting cuckoos are 'income breeders' (Drent & Daan 1980). Cuckoo samples from April contained DNA of rove beetle, grasshopper and snipefly species. However, their suitability as alternative prey in colder springs depends on their relative resilience to low spring temperatures compared to Lepidoptera. Months of occurrence of Lasiocampidae in faecal samples reflected occurrence as prey in England as captured from photographs (Fig 3a). Most Lepidoptera species were detected in cuckoo faeces in months matching their larval period. However at least one species, brown silver-line *Petrophora chlorosata*, a species commonly found by

day roosting in bracken (Waring & Towsend 2017), was detected in weeks corresponding primarily to adult flight season (Fig. 3a), illustrating that cuckoos on Dartmoor may also predate adult moths. DNA associated with moth family Limacodidae was frequently detected during sequencing but neither of the two UK species in this classification is expected from distribution to occur on Dartmoor. An alternative explanation for occurrence of DNA of this lineage is that it originates from a member of the encompassing superfamily Zygaenoidea, the most common of which is the six-spot burnet *Zygaena filipendulae*, found in this study's photo analysis to occur (in adult or imago) as prey of juvenile cuckoos.

Our analysis of photographs of cuckoo prey successfully sourced images from a wide geographic distribution around Britain. This approach differed from a similar current project on Atlantic puffins *Fratercula arctica* (RSPB 2017) in that all images were already present online. Analysis of photographic records is subject to similar taxonomic bias to observational field studies which make up much of the literature on cuckoo diet. On this basis the scope of this analysis was limited to identifying large prey captured in images since 2003 and comparing this qualitatively to large prey documented in the literature (mainly Lepidoptera). The most frequent adult cuckoo prey items captured by photography were drinker *E. potatoria* and oak eggar *L. quercus*. These are among the largest and most identifiable caterpillars in Britain, and are perhaps more likely to be identified from photographic images. However, equally large and recognisable species previously reported in the cuckoo diet were absent or rare within our analysis. Most notably, *A. caja* (garden tiger), once a common and widespread species (Conrad *et al.* 2002, 2006) has a large distinctive larva documented as cuckoo prey, but the species occurred in this analysis at low frequency and only in Scotland (Fig. 3a). This species is suggested to have declined throughout its range as a result of climate-mediated effects of increased winter rainfall and warmer springs (Conrad *et al.* 2002). The poor diversity of taxa detected in adult diet from photographs is not purely explained by limited ability to identify species in photographs as recognisable, and previously common, cuckoo prey taxa were also absent.

From photographs, juvenile prey was predominantly cinnabar *T. jacobaeae* larvae over much of the cuckoos' pre-migration period (Fig. 3b). This species has a prolonged larval emergence period (Waring & Townsend 2017), but is reported to have declined by 67% in Britain between 1968 and 2007 (Fox *et al.* 2013), in part possibly due to widespread population control of its main larval foodplant common ragwort *Senecio jacobaea* (DEFRA 2007) which has also declined (Carey *et al.* 2008). The analysis of juvenile cuckoo prey items illustrates that lepidopteran prey are available to juvenile cuckoos well into October in Britain. Across the two age classes of cuckoo studied, the array of species identified in our photograph study largely constitutes a subset of the large, defensive and warningly-coloured species listed as cuckoo prey in the monograph by Wyllie (1981). This is in contrast to Lepidoptera detected in the sequencing study, in which previously unreported families of both macromoth and micromoth were detected, some in significant frequency. Scarcity of images of juvenile cuckoos with prey in Scotland and Wales may be due to juveniles spending less time in natal areas in the west and the north after becoming independent. Analysis of ringing data showed that juveniles in Britain overwhelmingly moved south and east, beginning in August (Seel 1977a). On this basis most photographs of juveniles in our analysis are probably birds in the early stages of migration. The prey of the young cuckoo in the natal area, both before and after independence, is an area in urgent need of research, with almost no information published on timing of independence of how long juveniles self-feed before dispersing. Juveniles in Britain were lower body masses than adults (1977b), suggesting they may be dependent on prey availability during early on migration in Britain for both additional growth and fuelling migration to Europe.

Conclusions

The taxa of highest frequency in the cuckoo diet as analysed in this study are likely to be found in greater abundance in semi-natural habitats than in improved or arable agricultural land, and some are already widely reported to be vulnerable to intensification of land-use. Distribution changes in a significant proportion of British moths and butterflies are concurrent with changes in land cover that indicate intensification towards more arable and urban land use has

had negative impacts on moth populations (Fox *et al.* 2014). Increased field sizes remove traditional boundaries reported to support considerable biomass of moths relative to field centres (Merckx *et al.* 2009). Conversion of grasslands to arable land, a shift to monoculture crops and removal of uncropped margin area largely or completely eliminates the food plants of Lepidoptera in fields. Oak eggar, fox moth *Macrothylacia rubi*, brown-tail moth *Euproctis chrysorrhoea*, and *Aglais* and *Vanessa* butterflies found in the cuckoo diet in the present study have foodplants commonly found in field margins and hedgerows often lost during field enlargement (or unsympathetically managed with cutting and flailing machinery). Spraying of agrochemicals such as pesticides in arable land is likely to impact ground feeding larvae (and other insects) in spring when machinery operates at low crop height, while in summer the raised height of spraying is more likely to impact hedgerow-feeding larvae that could include oak eggar, fox moth and brown-tail moth in this period (Dover 2019). Declines in moths and butterflies have been greatest in arable areas, (Dover 2019) but intensification of grasslands for pasture is also concluded to impact on moths. Drinker moths *E. potatoria* feed at the larval stage on a range of grasses and reeds (Waring & Townsend 2017) that are likely to be outcompeted or grazed out of improved grasslands by reseeding or high density stocking of grazers, respectively (Fuller 1987). Field margins and hedgerows in pastoral and arable farmland may constitute the only semi-natural habitat in the landscape, but many are poor quality and fragmented (Dover 2019). Annual or more frequent cutting of hedgerows and field margins prevents many moth species from completing a ful life cycle (Merckx *et al.* 2009). Hedgerows with trees and wide semi-natural field margins were associated with higher abundance of moths, most notably when these were widespread and connected within the landscape, suggesting prominence of continuous semi-natural habitat benefits moth abundance (Merckx & Macdonald 2015). Some species found in this study are more confined to heath and semi-natural grasslands by their larval food plants, such as heath rustic *Xestia agathina*, brown silver-line *P. chlorosata*, *Pseudoterpna sp.* and emperor moth *Saturnia pavonia*.

Orthoptera also show vulnerability to intensive land use practices. High fertilizer application, mowing and high-density livestock grazing are associated with poor

orthopteran diversity and abundance (Chisté *et al.* 2016), and conversely orthopteran populations are relatively abundant and diverse in more semi-natural and less intensively managed areas of agricultural landscapes (Marini *et al.* 2008, Rodríguez & Bustamante 2008, Weking *et al.* 2016). Many species of Orthoptera lay eggs into soil, including common green grasshopper *O. viridulus* recorded in this study (Benton 2012) therefore cultivation such as ploughing may have survival impacts on eggs and early instars.

Overall, through use of novel techniques the present study has confirmed the importance of lepidopteran larvae, caterpillars, to the diet of adult and juvenile cuckoos. However, importance has been newly highlighted for grasshoppers (Orthoptera) within the adult diet, previously well-reported as prey of new world cuckoos. The early instars of grasshopper, and smaller invertebrates such as Diptera, are suggested here to be an alternative or supplementary prey taxon to caterpillars during early spring when cuckoos first arrive at breeding grounds and may be feeding to achieve breeding condition. The positive association of large moths and Orthoptera with semi-natural habitats suggest prey availability may be a major factor in the habitat associations of common cuckoos.

References

Abbey, G. (1909) *The Balance of Nature and Modern Conditions of Cultivation*. London: G. Routledge & Sons.

Armitage, J. S. (1978) Feeding methods and prey of the cuckoo. *British Birds* 71: 590–590.

Barrett, R. T., Camphuysen, K., Anker-Nilssen, T., Chardine, J. W., Furness, R. W., Garthe, S., Huppop, O., Leopold, M. F., Montevecchi, W. A., and Veit, R. R. (2007) Diet studies of seabirds: a review and recommendations. *ICES Journal of Marine Science* 64(9): 1675–1691.

Beal, F. E. L. (1898) 'The food of cuckoos'. In Beal, F. E. L. and Judd, S. D. (eds), 'Cuckoos and shrikes in their relation to agriculture', *Bull. U.S. Dept. Agric.* 9: 7-14.

Benton, T. (2012). *Grasshoppers and Crickets (Collins New Naturalist Library, Book 120)*. HarperCollins UK.

Blackburn, J., Austin, G., and Clark, J. (2006) Playback calls. *Ringers' Bulletin* 11(12): 114–115.

Bottomley, J. B., and Bottomley, S. (1975) Cuckoos photographed feeding on magpie moth caterpillars. *British Birds* 68(12): 514–514.

Chance, E. P. (1940) *The truth about the cuckoo*. London: Country Life.

Chaput, D. unpublished (2017) CTAB / EDTA DNA extraction protocol for filters. *BBSRC/DBT/Newton Aquaculture Project*, Exeter.

Chisté, M. N., Mody, K., Gossner, M. M., Simons, N. K., Köhler, G., Weisser, W. W., and Blüthgen, N. (2016) Losers, winners and opportunists: How grassland land-use intensity affects orthopteran communities. *Ecosphere* 7(11): e01545.

Clare, E. L., Barber, B. R., Sweeney, B. W., Hebert, P. D. N., and Fenton, M. B. (2011). Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Molecular Ecology* 20(8): 1772-1780.

Collinge, W. E. (1925) *The food of some British Wild Birds*. York: Yorkshire Herald Newspaper Company Ltd.

Condry, W. M. (1966) *The Snowdonia National Park*, Collins, London

Conrad, K. F., Woiwod, I. P., and Perry, J. N. (2002) Long-term decline in abundance and distribution of the garden tiger moth (*Arctia caja*) in Great Britain. *Biological Conservation* 106(3): 329–337.

Conrad, K. F., Warren, M. S., Fox, R., Parsons, M. S., and Woiwod, I. P. (2006) Rapid declines of common, widespread British moths provide evidence of an insect biodiversity crisis. *Biological Conservation* 132(3): 279–291.

Crawshaw, K. R. (1963) Juvenile Cuckoo's method of feeding upon Cinnabar Moth caterpillars. *British Birds* 56: 28–28.

Davies, M. (1987) Grasshoppers, Crickets and Bush-Crickets in Devon. *Nature In Devon* 8: 45–64.

Davis, P. R. K. (1996) *The cuckoo on Exmoor and its meadow pipit host*. Exmoor Natural History Society.

Dement'ev, G. P., and Gladkov, N. A. (1966) *Birds of the Soviet Union, Vol. 1*. (Dement'ev, G. P. and Gladkov, N. A., Eds.). Jerusalem: Israel Programme for Scientific Translations.

Denerley, C., Redpath, S. M., van der Wal, R., Newson, S. E., Chapman, J. W., and Wilson, J. D. (2018) Breeding ground correlates of the distribution and decline of the Common Cuckoo *Cuculus canorus* at two spatial scales. *Ibis*. doi:10.1111/ibi.12612.

Di Minin, E., Tenkanen, H., and Toivonen, T. (2015) Prospects and challenges for social media data in conservation science. *Frontiers in Environmental Science* 3: 63.

Dover, J.W. ed., (2019). *The Ecology of Hedgerows and Field Margins*. Routledge.

Drent, R. H., and Daan, S. (1980). The prudent parent: energetic adjustments in avian breeding. *Ardea* 68: 225-252.

Droscher, L. (1988) A study on radio-tracking of the European cuckoo (*Cuculus canorus canorus*). *Proc, Int. 100. DO-G Meeting, Current Topis Avian Biol., Bonn* 1988: 187–193.

Fox, R., Parsons, M.S., Chapman, J.W., Woiwod, I.P., Warren, M.S. and Brooks, D.R. (2013) *The State of Britain's Larger Moths 2013.* Butterfly Conservation and Rothamsted Research, Wareham, Dorset, UK

Essex, S., and Williams, A. (1992) Ecological effects of afforestation: a case study of Burrator, Dartmoor. *Applied Geography* 12(4): 361–379.

Fox, R., Oliver, T. H., Harrower, C., Parsons, M. S., Thomas, C. D., and Roy, D. B. (2014) Long-term changes to the frequency of occurrence of British moths are consistent with opposing and synergistic effects of climate and land-use changes. *Journal of Applied Ecology* 51(4): 949–957.

Fuller, R. M. (1987) The changing extent and conservation interest of lowland grasslands in England and Wales: A review of grassland surveys 1930–1984. *Biological Conservation* 40(4): 281–300.

Harris, S. J., Massimino, D., Gillings, S., Eaton, M. A., Noble, D. G., Balmer, D. E., Procter, D., and Pearce-Higgins, J.W. Woodcock, P. (2018) *The Breeding Bird Survey 2017. BTO Research Report 706*. Thetford.

Hebert, P. D., Stoeckle, M. Y., Zemlak, T. S., and Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS biology* 2(10): e312.

Hewson, C. M., Thorup, K., Pearce-Higgins, J. W., and Atkinson, P. W. (2016) Population decline is linked to migration route in the Common Cuckoo. *Nature Communications* 7: 12296.

Ishizawa, J., and Chiba, S. (1966) Food analysis of four species of cuckoos in Japan. *Miscellaneous Reports of Yamashima Institute of Ornithology* 4: 302–326.

Jacobsen, L. B., Jensen, N. O., Willemoes, M., Hansen, L., Desholm, M., Fox, A. D., Tøttrup, A. P., and Thorup, K. (2017) Annual spatiotemporal migration schedules in three larger insectivorous birds: European nightjar, common swift and common cuckoo. *Animal Biotelemetry* 5(1): 4.

Jeawak, S. S., Jones, C. B., and Schockaert, S. (2017) Using Flickr for characterizing the environment : an exploratory analysis. In *13th International Conference on Spatial Information Theory (COSIT 2017)*. doi:10.4230/LIPIcs.COSIT.2017.21.

Kerr, K. C., Lijtmaer, D. A., Barreira, A. S., Hebert, P. D., and Tubaro, P. L. (2009). Probing evolutionary patterns in Neotropical birds through DNA barcodes. *PLoS One* 4(2): e4379.

King, R. A., Symondson, W. O. C. and Thomas, R. J. (2015) 'Molecular analysis of faecal samples from birds to identify potential crop pests and useful biocontrol agents in natural areas', *Bulletin of Entomological Research* 105 (3): 261–272.

Korzun, L. P., Erard, C., Gasc, J. P., and Dzerzhinsky, F. J. (2003) Biomechanical features of the bill and and jaw apparatus of cuckoos, turacos and the hoatzin in relation to food acquisition and processing. *Ostrich - Journal of African Ornithology* 74(1–2): 48–57.

Lack, D. L. (1968). *Ecological adaptations for breeding in birds*. Methuen, London.

Li, W., and Godzik, A. (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22(13): 1658–1659.

Lowe, P. R. (1943) Some notes on the anatomical differences obtaining between the Cuculidae and the Musophagidae, with special reference to the specialization of the oesophagus in *Cuculus canorus* Linnaeus. *Ibis* 85: 490–515.

Marini, L., Fontana, P., Scotton, M., and Klimek, S. (2008) Vascular plant and Orthoptera diversity in relation to grassland management and landscape composition in the European Alps. *Journal of Applied Ecology* 45(1): 361–370.

Massimino, D., Woodward, I.D., Hammond, M.J., Harris, S.J., Leech, D.I., Noble, D.G., Walker, R.H., Barimore, C., Dadam, D., Eglington, S.M., Marchant, J.H., Sullivan, M.J.P., Baillie, S.R. and Robinson, R. A. (2017) *BirdTrends 2017: trends in numbers, breeding success and survival for UK breeding birds. Research Report 704.* Thetford.

McAtee, W. L. (1917) The shedding of the stomach lining by birds, particularly as exemplified by the Anatidæ. *The Auk* 34(4): 415–421.

McAtee, W. L. (1906) The Shedding of the Stomach Lining by Birds. *The Auk* 23(3): 346–346.

Merckx, T., Feber, R. E., Dulieu, R. L., Townsend, M. C., Parsons, M. S., Bourn, N. A. D., Riordan, P., and Macdonald, D. W. (2009) Effect of field margins on moths depends on species mobility: Field-based evidence for landscape-scale conservation. *Agriculture, Ecosystems and Environment* 129(1–3): 302–309.

Merckx, T. and Macdonald, D. W. (2015) ‘Landscape-scale conservation of farmland moths’, in Macdonald, D. W. and Feber, R. E. (eds) *Wildlife Conservation on Farmland. Volume 1: Managing for Nature on Lowland Farms*. Oxford University Press.

Nakamura, H., and Miyazawa, Y. (1997) Movements, space use and social organisation of radio tracked common cuckoos during the breeding season in Japan. *Japanese Journal of Ornithology* 46: 23–54.

Noakes, P. (2013) Catching cuckoos. *BTO Ringing News* 13(1): 11–11.

Pompanon, F., Deagle, B. E., Symondson, W. O., Brown, D. S., Jarman, S. N., and Taberlet, P. (2012) Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology* 21(8): 1931–1950.

Ratnasingham, S., and Hebert, P. D. N. (2007) BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* 7(3): 355–364.Richards, D. R., and Friess, D. A. (2015) A rapid indicator of cultural ecosystem service usage at a fine spatial scale: Content analysis of social media photographs. *Ecological Indicators* 53: 187–195.

Rose, L. N. (1982) Breeding ecology of British pipits and their Cuckoo parasite. *Bird Study* 29(1): 27–40.

RSPB (2017) *Introducing Project Puffin*, *Saving Species*. Available at: http://ww2.rspb.org.uk/community/ourwork/b/biodiversity/archive/2017/06/01/introducing-project-puffin.aspx.

Rodríguez, C., and Bustamante, J. (2008) Patterns of Orthoptera abundance and lesser kestrel conservation in arable landscapes. *Biodiversity and Conservation* 17: 1753–1764.

Seel, D. C. (1977a) Migration of the northwestern European population of the cuckoo *Cuculus canorus*, as shown by ringing. *Ibis* 119(3): 309–322.

Seel, D. C. (1977b) Trapping Season and Body Size in the Cuckoo. *Bird Study* 24(2): 114–118.

Simmons, R. E., Avery, D. M., and Avery, G. (1991). ‘Biases in diets determined from pellets and remains: correction factors for a mammal and bird-eating raptor.’ J. Raptor Res 25(3): 63-67.

Thorup, K., Tøttrup, A. P., Willemoes, M., Klaassen, R. H. G., Strandberg, R., Vega, M. L., Dasari, H. P., Araújo, M. B., Wikelski, M., and Rahbek, C. (2017) Resource tracking within and across continents in long-distance bird migrants. *Science Advances* 3(1): 1–11.

UKmoths.org.uk (2018) *Species accounts*. Available at: https://ukmoths.org.uk/systematic-list/.

Vega, M. L., Willemoes, M., Thomson, R. L., Tolvanen, J., Rutila, J., Samaš, P., Strandberg, R., Grim, T., Fossøy, F., Stokke, B. G., and Thorup, K. (2016) First-Time Migration in Juvenile Common Cuckoos Documented by Satellite Tracking. (Avilés, J. M., Ed.)*PLOS ONE* 11(12): e0168940.

Vogl, W., Taborsky, B., Teuschl, Y., Taborsky, M., and Honza, M. (2004) Habitat and space use of European cuckoo females during the egg laying period. *Behaviour* 141(7): 881–898.

Waring, P., and Townsend, M. (2017) *Field guide to the moths of Great Britain and Ireland*. Bloomsbury Publishing.

Weking, S., Kämpf, I., Mathar, W., and Hölzel, N. (2016) Effects of land use and landscape patterns on Orthoptera communities in the Western Siberian forest steppe. *Biodiversity and Conservation* 25(12): 2341–2359.

Willemoes, M., Strandberg, R., Klaassen, R. H. G., Tøttrup, A. P., Vardanis, Y., Howey, P. W., Thorup, K., Wikelski, M., and Alerstam, T. (2014) Narrow-front loop migration in a population of the common cuckoo *Cuculus canorus*, as revealed by satellite telemetry. *PloS one* 9(1): e83515.

Wyllie, I. (1981) *The Cuckoo*. London: Batsford.

Ying H., Beifang N., Ying G., Limin F., and Weizhong L. (2010) CD-HIT Suite: a web server for clustering and comparing biological sequences. *Bioinformatics* 26(5): 680–682.

Zeale, M. R. K., Butlin, R. K., Barker, G. L. A., Lees, D. C., and Jones, G. (2011) Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources* 11(2): 236–244.

6. Temporal and land-use trends in key prey moth species of cuckoo *Cuculus canorus* in Devon, from historic moth trapping data

Abstract

In Britain the common cuckoo *Cuculus canorus* shows a positive long-term trend in breeding abundance in semi-natural habitats compared to significant declines in other formerly frequent breeding habitats. Moth species that have historically been recorded as prey of the cuckoo have collectively shown similar changes in adult abundance between habitats, with positive trends in semi-natural habitats contrasting with declines in woodland, arable land and improved grassland. This suggests that habitat-specific temporal trends in cuckoo abundance may relate to prey population change. Analysis of variation in moth occurrence at the species level may be valuable, especially in a study area where molecular diet analysis has been carried out on cuckoos. In this study, volunteer light trapping and Rothamsted Insect Survey data from Devon, UK were used to analyse 43-year temporal trends, and 13-year temporal and land-use based trends, in adult occurrence of moth species taken as prey at larval stages by the cuckoo. Land use types were lowland areas which have relatively higher influence from intensive agriculture, upland areas of extensive semi-natural grass and heath, and intermediate buffer areas. Presence data for common cuckoo from standardized bird surveys were analysed over an identical study area to allow their land-management pattern of occurrence to be compared with those of prey moths. 'Upland' squares as defined in the study were distinctive in their extensive heath and semi-natural grassland cover and low arable land cover. Cuckoo occurrence was significantly higher in upland squares than in lowland or buffer squares. Only four of the fifteen study moth species displayed differences in temporal trend between land use types. One species was cinnabar moth *Tyria jacobaeae,* a common prey species of juvenile cuckoos, which increased in upland study areas and may have declined in lowlands and upland edge (buffer) due to increased control of their larval foodplant. An additional three species displayed greater rate of occurrence in uplands, including drinker moth *Euthrix potatoria* frequently taken as cuckoo

prey on Dartmoor and across Britain as shown in chapter 5. The increments of increased probability of cuckoo occurrence in buffer and upland squares most closely resembled those of a group of three species including drinker moth. Overall, occurrence of study moth species showed variable relationships with time and land-use, and some species cited as cuckoo prey showed more negative temporal trends in uplands. Moth species also showed variable long term trends within a standardised trapping dataset but those declining included two significant or formerly significant cuckoo prey. The study findings suggest concurrent relationships with land use between cuckoos and a subset of prey species, as in previous larger scale studies, but also suggest that knowledge of regional or local prey species is important for prioritising among prey populations showing variable trends; and this requires fine-scale diet studies.

Introduction

The common cuckoo *Cuculus canorus* is in decline in Britain (Harris *et al.* 2018) and in several European countries (PECBMS 2019). The national trend of declining abundance in Britain masks significant variation between habitat types. Breeding abundance change since 1995 is significantly more positive in semi-natural grass and heath than in other common breeding habitats (Massimino *et al.* 2017). While birds breeding in different regions of Britain have different migratory routes (Hewson *et al.* 2016), it is not yet confirmed that these differences are significantly correlated with breeding habitat. Cuckoos from all studied breeding regions spend the northern hemisphere winter in similar regions and habitat in sub-Saharan Africa, and migrate north by similar routes, as found from satellite tracking (Willemoes *et al.* 2014, Hewson *et al.* 2016). Inter-habitat variation in breeding trend may therefore relate to breeding ground factors more than carry-over effects from non-breeding areas.

The breeding ground diet of the cuckoo has not received significant study since before the onset of major decline in around 1985 (Massimino *et al.* 2017, PECBMS 2019). From observational field study, and stomach content analyses, the main prey of the cuckoo has been suggested to be large larvae of moths and butterflies (Lepidoptera). This is supported additionally by anatomical

studies which suggest that adaptations in the jaw and bill (Korzun *et al.* 2003), and stomach (McAtee 1906), have arisen to aid prolonged handling of large and physically or chemically defensive prey. On the basis that large Lepidoptera larvae are of high importance in the cuckoo diet, there is cause for concern as many species of this order are already reported to have declined in abundance and distribution (Conrad *et al.* 2006). More recent analysis of long-term trapping of moths by the Rothamsted Insect Survey shows that temporal trends in adult abundance in the UK have been more negative for species cited as prey of the cuckoo (Wyllie 1981) than for other species (Denerley *et al.* 2018). Analysing population change in known cuckoo prey species in relation to land cover surrounding Rothamsted Insect Survey traps shows declines in woodland, improved grassland and arable habitats but significant increases in semi-natural habitats (Denerley *et al.* 2018). These habitat-specific trends closely reflect those previously reported for the cuckoo from standardised monitoring surveys of breeding bird abundance (Massimino *et al.* 2017). Food supply may therefore explain differences in cuckoo population trend between regions and habitats, and declining moth numbers may have contributed to driving long term cuckoo decline in Britain and Europe.

Adult cuckoo prey in the present study area of Dartmoor National Park, Devon, UK, were analysed by DNA sequencing of prey remains in faeces in the previous chapter. This is a novel methodology for cuckoo diet analysis, and identification of prey from DNA means biases towards larger and harder-bodied prey inherent to field observation or faecal microscopy were avoided. The analysis confirmed the importance of large Lepidoptera larvae in terms of percentage of all faecal samples in which these were detected. Retraction in cuckoo distribution is evident in the county of Devon (Beaven & Lock 2016). Analysis of successive county bird atlases showed that recent cuckoo occupancy is negatively associated with agricultural land use when previously there was no significant relationship between occupancy and land use type (Denerley 2014). Presence-absence surveys showed positive association between cuckoos and semi-natural habitat cover (Denerley 2014). Because inter-habitat variation in population change is similar between cuckoos and moth species whose larvae ar e documented as cuckoo prey (Denerley *et al.*

2018), the present study hypothesised that changes in populations of key moth species may explain reduction of cuckoo occurrence in the lowlands and retention in the uplands in Devon. In the present study I analysed adult moth captures and adult cuckoo occurrence in Devon across areas of upland and lowland land use. Temporal and land-use based variation in reporting rate of moth species were determined from records obtained during light-trapping, carried out by both volunteer individuals and groups and a national standardised survey (Rothamsted Insect Survey).

Success of light trapping, in terms of abundance and diversity captured, varies with time of night, duration and weather conditions and therefore presents a greater challenge for standardisation than methods such as field transects as can be readily applied for butterflies and day-flying moths (New 2004). However, light trapping is commonly and widely deployed both for for surveying moth diversity and as a pastime for many naturalists, and so often provides the most substantial body of data from which to make assessments of changes in diversity, assemblage or rate of occurrence (New 2004). Rate of occurrence at light traps has, for example, been used to estimate changes in phenotypic ratio of pale and melanistic morphs of peppered moth *Biston betularia* (Grant *et al.* 1996). Rates at which species are reported within simple lists have been shown to significantly correlate with more intensively collected survey data (Roberts *et al.* 2007). Reporting-rate based methods are therefore suggested as a useful monitoring tool in scenarios where resources are insufficient for in depth surveys of abundance. While Roberts *et al.* (2007) referred mainly to resource-poor regions, list-based data are also useful in instances where more intensively standardised data is lacking, for taxa of high and immediate conservation interest, provided sources of bias and limitations are considered.

From the data, the following questions were addressed: i) What are the long-term trends in occurrence probability at light traps in Devon of moth species cited to be predated as larvae by common cuckoos (Wyllie 1981)? ii) How does cover of specific habitat types vary between lowland, upland and buffer land use categories used in the present study? iii) How do differences in cover of habitats between the lowland, upland and buffer moth trapping areas compare to habitat differences between entire lowland, upland and buffer 10 km squares? iv) How

does adult capture probability for ‘cuckoo prey’ moth species (at light traps and Rothamsted Insect Survey traps) vary between land management categories in Devon, and do temporal trends in adult abundance differ between these broad land-management types? v) Does the cuckoo show different rate of occurrence between these land-management types, and are these differences similar to those seen in any prey moth species trapped over a similar time period? Based on the findings of recent analyses of Rothamsted Insect Survey data and land-use (Denerley *et al.* 2018), cuckoo prey moth species were predicted to show more negative population change in lowland areas which have greater presence of intensive agricultural land-use, relative to upland areas where semi-natural habitats are more prevalent. I also predicted cuckoo presence to be significantly more probable in upland squares due to prevalence of semi-natural habitats and scarcity of intensive agricultural land management. It was predicted that land-management differences in cuckoo presence-absence would correlate with those of the most frequent prey taxon, lasiocampid moths.

Methods

Study area and moth data

Moth species lists submitted to Devon Moth Group from the Rothamsted Insect Survey sites, and from individuals and groups undertaking light trapping of adult moths, were accessed for the 17 10 km British National Grid squares lying within or overlapping the boundary of the designated area of Dartmoor National Park in Devon, UK (SX47, SX48, SX49, SX55, SX56, SX57, SX58, SX59, SX65, SX66, SX67, SX68, SX69, SX76, SX77, SX78 and SX79). The park is designated predominantly for its extensive rural land-uses of semi-natural grassland and heathlands, and presents a landscape with significant presence of these habitats and less influence of arable, urban or intensively improved habitats. From the remainder of British National Grid 10 km grid squares overlying the county of Devon, I randomly selected an equal sized sample of 17 ‘lowland’ squares from which moth records of known date and trapping method were available, for comparison, having first excluded from this pool all squares

where more than 50% of their area comprised i) sea ii) Exmoor National Park or ii) land in other counties.

Examining satellite imagery in Google Earth (Google 2018) of land cover within the resultant 34 10 km squares, the physical extent of raised, unenclosed, open (non-wooded) habitat varied among squares designated as Dartmoor National Park according to the administrative boundary alone, and two squares categorised as lowland were located at the edge of Exmoor National Park uplands. An intermediate land management 'buffer' category was therefore created to include all 10 km squares with visible cover of up to 25% unenclosed open habitat according to satellite imagery in Google Earth. Seven squares at the periphery of Dartmoor National Park area and two squares at the periphery of Exmoor National Park

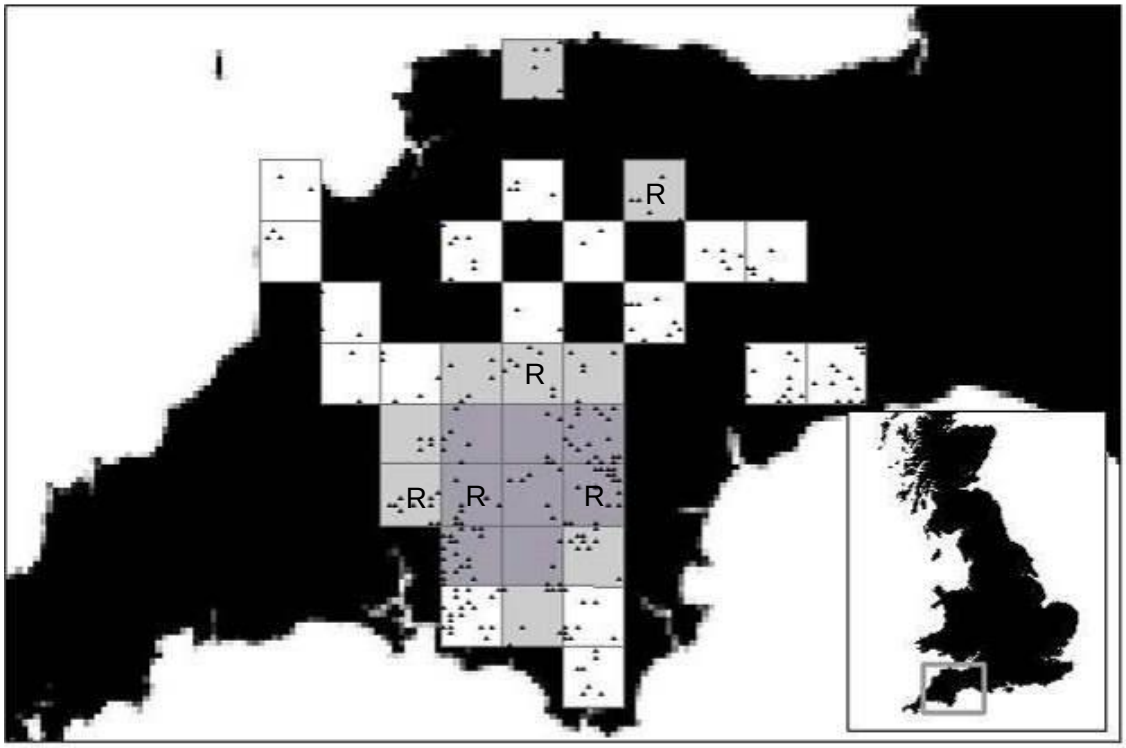


Figure 1. Distribution of British National Grid 10 km squares in Devon (white = lowland, pale grey = buffer, dark grey = core upland) used in analysis of moth captures and cuckoo survey data between land management types. Squares marked R contain Rothamsted Insect Survey traps used in analysis of long-term change in capture rate. Filled triangles ▲ are Rothamsted Insect Survey traps and other light-trapping sites (n = 234) included in analysis of moth capture rates between land management types.

were reclassified as buffer squares. Two squares at the periphery of the Dartmoor National Park area with no visible unenclosed open habitat were reclassified as lowland. This reclassification resulted in 17 lowland squares, 9 buffer squares and 8 core upland squares (Fig. 1).

From moth records, only those of adult moths which originated from intentional light-trapping (c.f. moths counted at outdoor light fixtures) were included. Records which combined light trapping with active searching or field observation were not included. Only trapping sessions with an exact date were included. All species lists were considered to represent complete lists. From species lists at each date and site (hereafter 'trapping sessions'), presence (1) or absence (0) was recorded for each moth species recorded in the diet of cuckoos by Wyllie (1981), excluding hawk moths Sphingidae which are not named at species level in this publication. Across our 34 selected 10 km squares, Rothamsted Insect Survey data was available from traps in five of the selected squares (one trap per square) for years 1971-2013: SS82, SX47, SX57, SX69 and SX77. These squares were all categorised as buffer or core upland (Fig. 1). The traps in SS82, SX47, SX69 and SX77 were similarly located at edge habitat between broadleaf woodland and enclosed agricultural land, in the vicinity of well-wooded river valleys, hilly enclosed agricultural land and rural human habitation. The trap in SX57 was in contrast located beside a television aerial at a hill summit, in the vicinity of predominantly unenclosed grass moor, plus suburban human habitation, enclosed grassland and mixed woodland. Light trapping data from individual volunteers and moth groups, submitted to Devon Moth Group, was available across all 34 10 km squares. Compared to Rothamsted Insect Survey traps which carried a tungsten bulb, were run on a standardised often nightly basis, and were lethal to the individuals trapped, Devon Moth Group data derived from a range of mercury vapour and occasionally actinic light sources, were run at varying times of night and months of the year, and were non-lethal. Because information on light source type and power, or weather were not consistently provided over the period of the historic data, the proxy of total number of species captured per session (species list-length) was required as a proxy for total catch as an assumed function of weather, trapping timing and method.

Table 1. Relationship of Land Cover Map 2007 target classes (Morton *et al.* 2011) to habitat variables for the present study

LCM2007 target class	Allocation to habitat type current study
1. Broadleaved woodland	Broadleaf woodland BROADLEAF
2. Coniferous woodland	Coniferous woodland CONIFEROUS
3. Arable and horticulture	Arable land ARABLE
4. Improved grassland	Improved grassland IMPROVED
5. Rough grassland 6. Neutral grassland 7. Calcareous grassland 8. Acid grassland 9. Fen, marsh and swamp	Semi-natural grassland SEMINAT
10. Heather 11. Heather grassland 12. Bog	Heathland HEATH
16. Freshwater	Freshwater FRESHWATER
13. Montane habitats 14. Inland rock 15. Saltwater 17. Supra-littoral rock 18. Supra-littoral sediment 19. Littoral rock 20. Littoral sediment 21. Saltmarsh 22. Urban 23. Suburban	'Non-vegetated' habitats UNVEG

Habitat data

The 1km Percentage Target Class dataset from the Land Cover Map 2007 survey (Morton *et al.* 2011) (hereafter LCM2007) was used to determine percentage cover of all habitat types in the study area. This comprises 23 raster spatial datasets of cell size 1 x 1 km, aligned with the 1 km British National Grid co-ordinate system, with each cell carrying percentage cover data for one of 23 'target' habitat classes (Table 1). Habitat classification is carried out by a mixture of human and machine-based recognition of vegetation and other

substrates according to reflectance of different bands of the electromagnetic spectrum as recorded by satellite imaging (Morton *et al.* 2011). Grid-references of sites included in the moth dataset were accurate to at least the 1 km British National Grid square. For each distinct 1 km square providing trapping data, I derived the central 12-figure numerical X and Y values used for this co-ordinate system in GIS software (e.g. mid point of grid square SS2117 = 221500, 117500). The list of 1 km British National Grid and XY co-ordinates was imported to ArcMap 10.1 (ESRI, Redlands USA) and converted to a vector point spatial dataset, displayed geographically over the LCM2007 habitat rasters. The 34 10 km squares that constituted the study area were added as a vector polygon dataset to the GIS. Squares were digitised by hand and coordinates of each vertex were corrected to exact values in sketch properties.

To complete a 1 km resolution habitat dataset across the spatial extent of the moth trapping area, a Multiple Values to Point process was run, to join the cell value (percent cover) of each of the 23 LCM2007 raster layers, to the vector point where moth trapping was conducted. Following this, cover for some variables was then summed to generate percentage cover for aggregate habitat variables used in the present analysis as described in Table 1. Details were added including 10 km British National Grid square identity within which each trapped 1 km square lay, and that 10 km square's assigned land management category (lowland, buffer, core upland), to allow comparison of (moth trapping) habitat between land management categories [analysis (ii) below].

The mean cover for the 'moth trapping area' of each 10 km square was obtained by production of mean values per 10 km square from the relevant 1

km square data. In order to also obtain mean cover of each habitat across the full extent of each 10 km square (this full extent included all moth-trapped 1 km squares), and compare this with the habitat covered by moth trapping effort, the vector polygons of the 34 squares in the GIS were used as zones for 23 Zonal Statistics as Table processes, one for each LCM2007 habitat class raster. These extracted mean percentage cover of each habitat class per 10 km square into an individual table. The table for habitat class 1 was joined in turn with the remaining 22 tables, unified by object ID (square identity), to collate mean covers of all habitat classes. Cover of some variables was summed to aggregated habitat classes (Table 1), and details of the land management category of each 10 km square (lowland, buffer, core upland) were added.

Cuckoo data

Counts of adult cuckoos in 2 x 2 km survey tetrads were obtained from the Devon bird atlas dataset maintained by DevonBirds. Adult birds of all species were counted during timed tetrad visits (TTVs) between 2007 and 2011 for the Atlas of birds in Britain and Ireland co-ordinated by the BTO (Balmer *et al.* 2013), or between 2011 and 2013 to complete coverage for the Devon county atlas, using identical field methodology (Beaven & Lock 2016). During the combined period, tetrads covering all of Devon county received at least two surveys each, one in April or May (early breeding season), and a second in June or July (late breeding season).

Statistical analysis

i) Long-term trends in capture probability of moth species in Rothamsted Insect Survey traps

In order to estimate long-term temporal change in occurrence of cuckoo prey species, I tested whether there was a linear relationship between year and logit probability of capture of each moth species cited to be cuckoo prey by Wyllie (1981), using trapping data from the Rothamsted Insect Survey for the period

1971-2013. I carried this out for all moth species trapped in 50 or more sessions across all years and traps (total n = 17,066 sessions at five trapping sites, each in a different 10 km square). For each species, statistical tests were carried out on a subset of the data which included only the range of months where the species was trapped in five or more sessions across all sites and years. This was designed to reflect the species' adult flight period in Devon. For final sample sizes and inventory of 10 km squares and months included in each species' model, see appendices. For each species I fitted a binomial logistic regression with the response variable Presence (1 or 0) and the covariates Year (1 - 43) and species-list length (to represent total catch size), and categorical factors Month and 10 km square identity as fixed effects. Variables in this initial model that were not significant at $P < 0.05$ were removed on an iterative basis according to largest probability value first, until only variables with significant effects were present in the model.

ii) How does cover of specific habitat types in moth trapping sites vary between broad land management categories?

In order to identify the differences in specific habitats between the broad land use types of upland, buffer and lowland defined by the present study, I tested whether cover of each aggregate habitat type defined in Table 1 significantly differed between 10 km squares with the three land use categories. For each aggregate habitat type e.g. broadleaf, the LCM2007 1 km resolution percentage cover (in each 1 km British National Grid square where moth trapping occurred), was the response variable in a general linear mixed model, with square category (lowland, buffer, core upland) included as a fixed factor, and 10 km square identity as a random factor. This analysis specifically compared cover of habitat in moth trapping sites between land-use types, rather than the overall landscapes of the different categories of 10 km squares included in the study. Analysis at the level of moth trapping area was appropriate as this was the study area used in analysis (iv) for testing differences in moth occurrence between land use types.

iii) How do differences in cover of habitats at the level of moth trapping area between land use types compare to habitat differences at the level of 10 km squares?

Analysis (ii) tested habitat differences between land use types in the moth-trapped extent of the study area. It was valuable to the present study to assess whether these differences are representative of differences at the wider 10 km square scale. In order to illustrate how habitat cover differed between entire 10 km squares categorised as upland, lowland and buffer, percentage habitat cover was calculated across the whole of each 10 km square included in the study. The 1 km resolution data from analysis (ii) was used to produce mean percentage cover of habitat on moth trapping sites per 10 km square. Habitat cover variation with land use type, as calculated by the two routes, was displayed as boxplots.

iv) How does adult capture probability for moth species vary between land management categories in Devon, and do temporal trends in adult abundance differ?

The key analysis of the present study was to test whether 'cuckoo prey' moth species have undergone different temporal trends in occurrence rate within the different land use types (lowland, upland, buffer), with the prediction that prey moth species would display more positive trends in occurrence rate in upland land use. In order to test variation and trend in moth capture probability with land use type, I modelled the effects of year, square category (lowland, core upland, buffer) and crucially the interaction term of these two variables, on logit probability of capture of each study moth species in a generalized linear mixed model (GLMM) with binomial error distribution and logit link. I used data from Rothamsted Insect Survey sessions and volunteer light trapping sessions for the years 2003-2016. Over this period data was consistently available from ten or more 10 km squares per year. I conducted this analysis for each moth species reported as cuckoo prey in Wyllie (1981) which had been captured as adults in 50 or more sessions across all sites and years. For each species, sessions were only included from calendar months where the species was trapped in five or more sessions across all sites and years, to represent the

adult flight season of the species in Devon. For final sample sizes, and inventory of months included in each species' model, see appendices. For each species a GLMM logistic regression was fitted, with the response variable Presence (1 or 0). Month, square category (upland, buffer, lowland), and trapping method (Rothamsted or small light trap) were included as fixed, categorical factors. Year, the interaction term between square category and year, the interaction between trapping method and year, and total species list length (to represent total catch size) were included as covariates. The interaction term between square category and year was included to test whether there was variation in temporal trend between land use categories. The interaction term between trapping method and year was included to test whether temporal trend varied between trap types, for example due to increased effectiveness of small light traps over time.The random effects were 1km square identity nested within 10km square identity. If fixed effects were found to be non-significant at $P < 0.05$, and were not part of a significant interaction term, they were removed stepwise from iterative subsequent reruns of the model in order of largest to smallest probability value, until only terms with significant effects were included.

v) Do cuckoos show different rate of occurrence between land-management types?

All tetrads in Devon received one visit in early (April and May) and late (June and July) breeding season in at least one year between 2007 and 2013. Visit length was either 1 or 2 hours. To determine the importance of this variation, I tested whether hours of effort in a TTV significantly correlated with number of cuckoos recorded, for tetrads where cuckoos were detected. Maximum count of cuckoo adults in a tetrad was tested as a response to number of hours (1 or 2) as a fixed factor in a general linear mixed model with poisson error distribution and tetrad identity as a random factor. Hours of effort was not significant in estimated effect (GLMM, two versus one hour estimate (log) = +0.0778 ± S.E. 0.141, $P = 0.582$) therefore I did not proceed to include total survey hours in subsequent analyses. Cuckoo presence in a tetrad was recorded as 1 if a cuckoo was found during either or both visits, and as 0 where a cuckoo was not recorded. To enable comparison of analytical outcomes for cuckoos and moths,

only bird atlas tetrads that overlapped with the moth-trapped area of the 34 10 km squares used in the present study (Fig. 1) were included in analysis. This study area comprised 195 tetrads. A mixed model logistic regression (a GLMM with binomial errors and a logit link function), was used to test presence (1 or 0) of cuckoos in tetrads as a response to land use category (upland, lowland, buffer) as a fixed categorical factor and 10 km square identity as a random effect.

In order to compare land-management associations of cuckoos and moths, the final selected model for each moth species from analysis (iii) was used to plot the estimated (logit) difference in capture probability in buffer squares versus lowland squares, against the estimated (logit) difference in upland versus lowland squares. For moth species with a significant interaction term between year and square category, the estimated difference in capture probability was calculated using year 7 (the midpoint of the modelled time series for moth captures). The estimated difference in encounter probability of cuckoo for period 2007-13 was plotted alongside those for moth species to allow direct comparison of cuckoo and prey species occurrence between land management categories.

All statistical analysis was conducted using R version 3.5.0. Mixed modelling was performed using R package *lme4* (Bates *et al.* 2015).

Results

Rothamsted Insect Survey data and volunteer light trapping data were accessed across all 34 selected 10 km squares, resulting in data from 5 Rothamsted trapping sites across 5 10 km squares (available for total period 1971 to 2013, total distinct trapping dates and sites i.e. sessions = 17066). Selecting a subset of years for which volunteer light-trapping data was available for 10 or more 10 km squares per year resulted in volunteer data from 229 1 km squares (hereafter ‘sites’), and the 5 Rothamsted trapping sites, for the period 2003-2016. In this period the total number sessions was 12588 (n = 6865 volunteer, n = 5723 Rothamsted survey).

i) Long-term trends in capture probability of moth species in Rothamsted Insect Survey traps

13 species were captured in 50 or more Rothamsted Insect Survey sessions across sites between 1971 and 2013. Of our study species, only antler moth *Cerapteryx graminis*, fox moth *Macrothylacia rubi*, white ermine *Spilosoma lubricipeda* and cinnabar *Tyria jacobaeae* were captured in the Rothamsted Insect Survey trap at the relatively exposed site in SX57 near open moorland, and most species models therefore included capture data from four traps. The subset of months included for each species model resulted in sample sizes typically between 3000 and 8000 trapping sessions over the study period.

Log-odds of capture of garden tiger *Arctia caja*, white ermine, cinnabar and winter moth *Operophtera brumata* declined significantly with year. Log-odds of capture for drinker *Euthrix potatoria*, antler moth, buff ermine *Spilosoma lutea* and early thorn *Selenia dentaria* significantly increased. No significant temporal trend in capture probability was detected for magpie moth *Abraxas grossulariata*, dotted border *Agriopis marginaria*, March moth *Alsophila aescularia*, buff-tip *Phalera bucephala* and fox moth (Table 2).

ii) How does cover of specific habitat types in moth trapping sites vary between broad land management categories?

Across 1 km grid squares where moth trapping was carried out in lowland, buffer and core upland squares, arable land cover was significantly lower in buffer than in lowland squares, and lower in core upland squares than in buffer squares. Lowland squares contained significantly lower cover of broadleaf woodland than buffer or upland squares, and upland contained significantly more cover of heath and semi-natural grassland than lowland or buffer squares. Square categories showed no significant difference in cover of coniferous woodland, improved grassland or freshwater habitats. Unvegetated habitat cover of buffer squares was intermediate and lay within the significant difference in cover of this habitat between core upland and lowland squares (Fig. 2a).

Table 2. Estimated effects of significant variables on log-odds of capture of adults of 13 moth species cited as larval prey of common cuckoo, at Rothamsted Insect Survey traps in Devon 1971-2013. Month effects listed are relative to calendar month preceding first month shown per species. 10 km square effects are relative to square SS82.

Garden tiger	Estimate (logit)	S.E.	*P*	
(Intercept)	-2.642	0.385	<0.0001	***
Year	-0.075	0.021	0.0004	***
Month = August	-0.752	0.302	0.0127	*
10km = SX47	-0.875	1.084	0.4194	
10km = SX69	1.362	0.703	0.0527	.
10km = SX77	-2.368	0.521	<0.0001	***
ListLength	0.055	0.009	<0.0001	***

Drinker	Estimate (logit)	S.E.	*P*	
(Intercept)	-5.051	0.356	<0.0001	***
Year	0.047	0.005	<0.0001	***
Month = July	2.959	0.260	<0.0001	***
Month = August	2.773	0.264	<0.0001	***
10km = SX47	-2.053	0.337	<0.0001	***
10km = SX69	-1.492	0.310	<0.0001	***
10km = SX77	-2.046	0.293	<0.0001	***
ListLength	0.044	0.004	<0.0001	***

Magpie moth	Estimate (logit)	S.E.	*P*	
(Intercept)	-5.284	0.459	<0.0001	***
Month = August	2.106	0.171	<0.0001	***
Month = September	-0.602	0.322	0.0620	.
10km = SX47	2.297	0.424	<0.0001	***
10km = SX69	0.490	0.440	0.2655	
10km = SX77	-1.573	0.437	0.0003	***
ListLength	0.073	0.006	<0.0001	***

(Year estimate non-significantly different from 0)

Dotted border	Estimate (logit)	S.E.	*P*	
(Intercept)	-4.831	0.599	<0.0001	***
Month = February	2.645	0.285	<0.0001	***
Month = March	2.356	0.283	<0.0001	***
Month = April	-0.116	0.312	0.7100	
Month = May	-8.595	0.832	<0.0001	***
10km = SX47	0.715	0.549	0.1920	
10km = SX69	-1.052	0.647	0.1040	
10km = SX77	0.569	0.539	0.2910	
ListLength	0.245	0.014	<0.0001	***

(Year estimate non-significantly different from 0)

March moth	Estimate (logit)	S.E.	*P*	
(Intercept)	-0.144	0.331	0.6636	
Month = March	-0.284	0.123	0.0213	*
Month = April	-2.549	0.175	<0.0001	***
10km = SX47	-1.625	0.345	<0.0001	***
10km = SX69	-2.282	0.408	<0.0001	***
10km = SX77	-1.735	0.328	<0.0001	***
ListLength	0.240	0.014	<0.0001	***

(Year estimate non-significantly different from 0)

Antler moth	Estimate (logit)	S.E.	*P*	
(Intercept)	-3.942	0.349	<0.0001	***
Year	0.046	0.009	<0.0001	***
Month = August	2.405	0.223	<0.0001	***
Month = September	0.452	0.318	0.1560	
10km = SX47	-2.272	0.443	<0.0001	***
10km = SX57	3.396	0.428	<0.0001	***
10km = SX69	-2.752	0.416	<0.0001	***
10km = SX77	-3.096	0.372	<0.0001	***
ListLength	0.039	0.007	<0.0001	***

Fox moth	Estimate (logit)	S.E.	*P*	
(Intercept)	-3.187	0.473	<0.0001	***
Month = June	-0.039	0.240	0.8698	
Month = July	-2.538	0.406	<0.0001	***
10km = SX47	-3.696	1.104	0.0008	***
10km = SX57	-0.306	1.116	0.7836	
10km = SX77	-1.164	0.493	0.0182	*
ListLength	0.052	0.007	<0.0001	***

(Year estimate non-significantly different from 0)

Winter moth	Estimate (logit)	S.E.	*P*	
(Intercept)	-0.404	0.354	0.2530	
Year	-0.021	0.004	<0.0001	***
Month = February	-2.822	0.150	<0.0001	***
Month = November	-3.035	0.144	<0.0001	***
Month = December	-0.234	0.110	0.0343	*
10km = SX47	0.952	0.393	0.0155	*
10km = SX69	-0.217	0.433	0.6155	
10km = SX77	1.024	0.356	0.0040	**
ListLength	0.244	0.021	<0.0001	***

Buff tip	Estimate (logit)	S.E.	*P*	
(Intercept)	-4.368	0.351	<0.0001	***

Month = June	1.662	0.234	<0.0001	***
Month = July	2.241	0.230	<0.0001	***
Month = August	-1.171	0.373	0.0017	**
10km = SX47	-0.062	0.310	0.8409	
10km = SX69	-1.017	0.339	0.0027	**
10km = SX77	-0.438	0.295	0.1383	
ListLength	0.041	0.003	<0.0001	***

(Year estimate non-significantly different from 0)

Early thorn	Estimate (logit)	S.E.	*P*	
(Intercept)	-2.431	0.193	<0.0001	***
Year	0.010	0.003	0.0002	***
Month = April	0.908	0.102	<0.0001	***
Month = May	-0.488	0.115	<0.0001	***
Month = June	-4.063	0.236	<0.0001	***
Month = July	0.078	0.119	0.5144	
Month = August	1.115	0.104	<0.0001	***
Month = September	-1.758	0.160	<0.0001	***
10km = SX47	-0.405	0.212	0.0567	.
10km = SX69	-2.058	0.238	<0.0001	***
10km = SX77	0.176	0.186	0.3445	
ListLength	0.071	0.003	<0.0001	***

White ermine	Estimate (logit)	S.E.	*P*	
(Intercept)	-0.664	0.186	0.0003	***
Year	-0.021	0.004	<0.0001	***
Month = June	1.486	0.102	<0.0001	***
Month = July	-0.759	0.127	<0.0001	***
Month = August	-4.367	0.459	<0.0001	***
10km = SX47	-0.986	0.250	<0.0001	***
10km = SX57	-4.008	1.033	0.0001	***
10km = SX69	-0.888	0.232	0.0001	***
10km = SX77	-2.196	0.206	<0.0001	***
ListLength	0.058	0.003	<0.0001	***

Buff ermine	Estimate (logit)	S.E.	*P*	
(Intercept)	-3.239	0.252	<0.0001	***
Year	0.010	0.004	0.0079	**
Month = June	3.153	0.174	<0.0001	***
Month = July	1.614	0.181	<0.0001	***
Month = August	-1.988	0.359	<0.0001	***
10km = SX47	-0.429	0.264	0.1047	
10km = SX69	-1.665	0.257	<0.0001	***
10km = SX77	-1.837	0.230	<0.0001	***
ListLength	0.057	0.003	<0.0001	***

Cinnabar	Estimate (logit)	S.E.	*P*	
(Intercept)	-3.782	0.733	<0.0001	***
Year	-0.072	0.019	0.0002	***
Month = July	-0.943	0.305	0.0020	**
10km = SX47	0.607	1.222	0.6192	
10km = SX57	0.768	1.248	0.5379	
10km = SX69	2.379	0.932	0.0107	*
10km = SX77	-0.303	0.819	0.7118	
ListLength	0.041	0.010	<0.0001	***

iii) How do differences in cover of habitats at the level of moth trapping area between land use types compare to habitat differences at the level of 10 km squares?

Comparing habitat cover across the moth trapping portion (Fig. 2a) and full extent (Fig. 2b) of 10 km grid squares, in lowland squares moth trapping sites contained lower arable land cover than in the wider 10km square. In buffer squares, arable land and semi-natural grassland were scarcer in 1km squares used for moth trapping than in the wider 10km square, while cover of broadleaf and coniferous woodland were higher in moth trapping 1km squares. In core upland squares, heath and semi-natural grassland cover in moth trapping 1km squares were scarcer than in the wider 10 km squares, while cover of improved grassland and broadleaf woodland were higher (Fig. 2b).

iv) How does adult capture probability for moth species vary between land management categories in Devon, and do temporal trends in adult abundance differ?

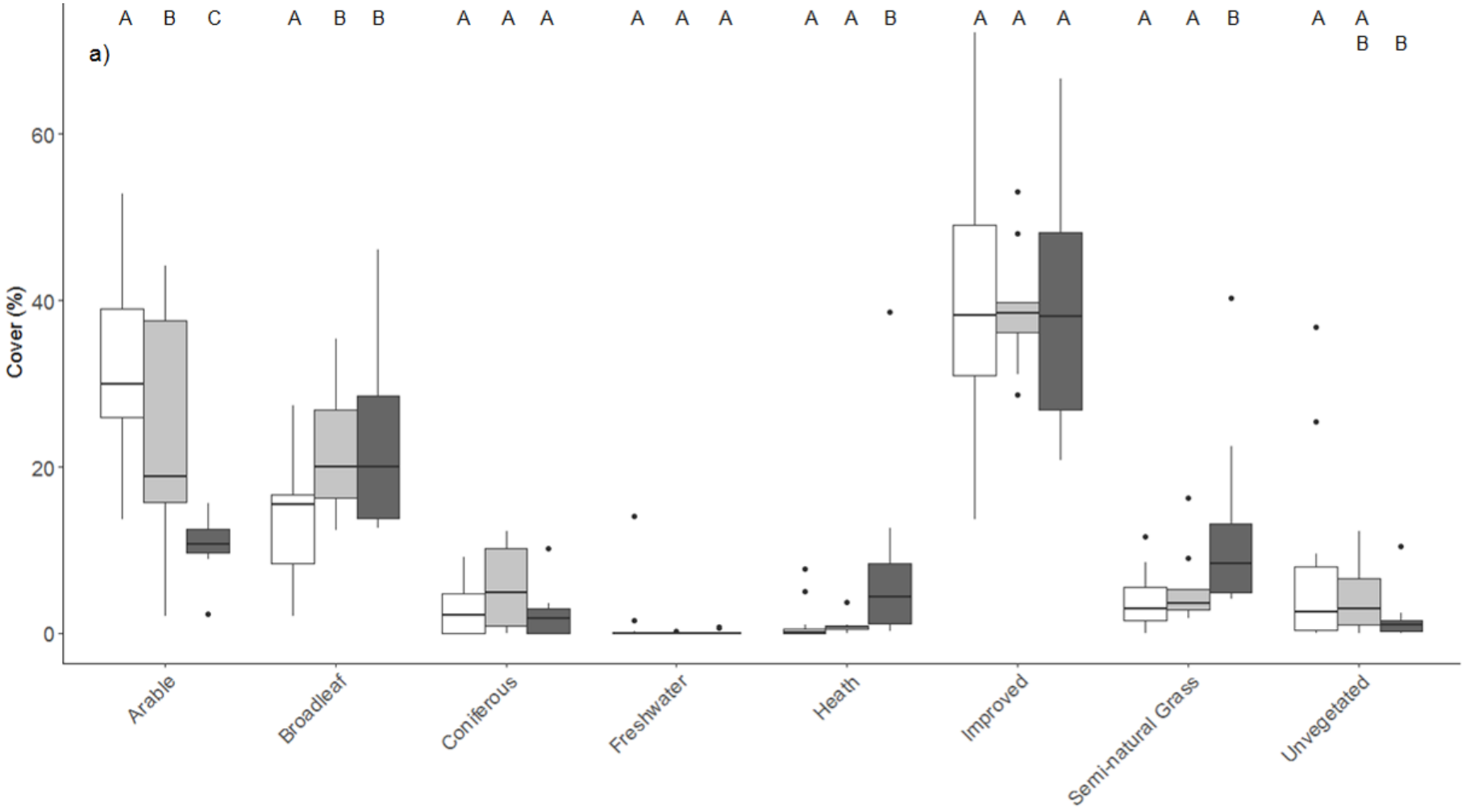
Models for capture probability trends between 2003 and 2016 of antler moth, cinnabar, early thorn, and magpie moth contained an interaction term between year and land use category (upland, lowland, buffer) that was statistically significant, suggesting different temporal trends in occurrence between land use types (Table 3). Antler and cinnabar moth occurrence showed a more positive temporal trend in upland land use squares, early thorn and magpie moth occurrence showed a more negative temporal trend in upland land use squares (Table 3) (Fig. 3).

Additionally, white ermine, drinker and winter moth capture probability were significantly higher in upland land use squares, with no significant difference in temporal trend between land use types. These species were respectively modelled to have positive, stable and negative temporal trends in occurrence. Lackey *Malacosoma neustria* capture probability was significantly lower in upland squares, with no significant difference in temporal trend between land use types (Table 3) (Fig. 3).

Capture probabilities of March moth, dotted border, buff ermine and buff-tip showed significant positive correlations with year with no significant difference between land management categories, but with significantly more negative temporal trends in occurrence at Rothamsted traps versus light traps (Table 3). Capture probability of garden tiger declined over time in all land use categories. Oak eggar *Lasiocampa quercus* and fox moth were modelled to have consistently low capture probability across years and land management categories (Table 3) (Fig. 3).

v) Do cuckoos show different rate of occurrence between land-management types?

Across the study area used for moth capture analysis, common cuckoo presence in 2 x 2 km tetrads between 2007 and 2013 was significantly higher in core upland 10 km squares than in lowland or buffer squares (general linear model, n = 195 tetrads, Fig. 4, logit estimate change in presence probability for core upland c.f. lowland = +2.200 ± S.E. 0.450, $P < 0.0001$, logit estimate change for buffer squares c.f. lowland = -0.134 ± 0.641, $P = 0.835$). The estimated logit differences, with standard errors, in cuckoo presence probability and in moth species' capture probability, for buffer squares and for upland squares relative to lowland squares, are plotted in Figure 5. Modelled differences in cuckoo presence in core upland and in buffer squares relative to lowland were most similar to drinker moth but this species showed a similar relationship to land use as white ermine and winter moth (Fig. 5).


a)
A B C
A B B
A A A
A A A
A A B
A A A
A A B
A A
B B
Cover (%)
0
20
40
60
Arable
Broadleaf
Coniferous
Freshwater
Heath
Improved
Semi-natural Grass
Unvegetated

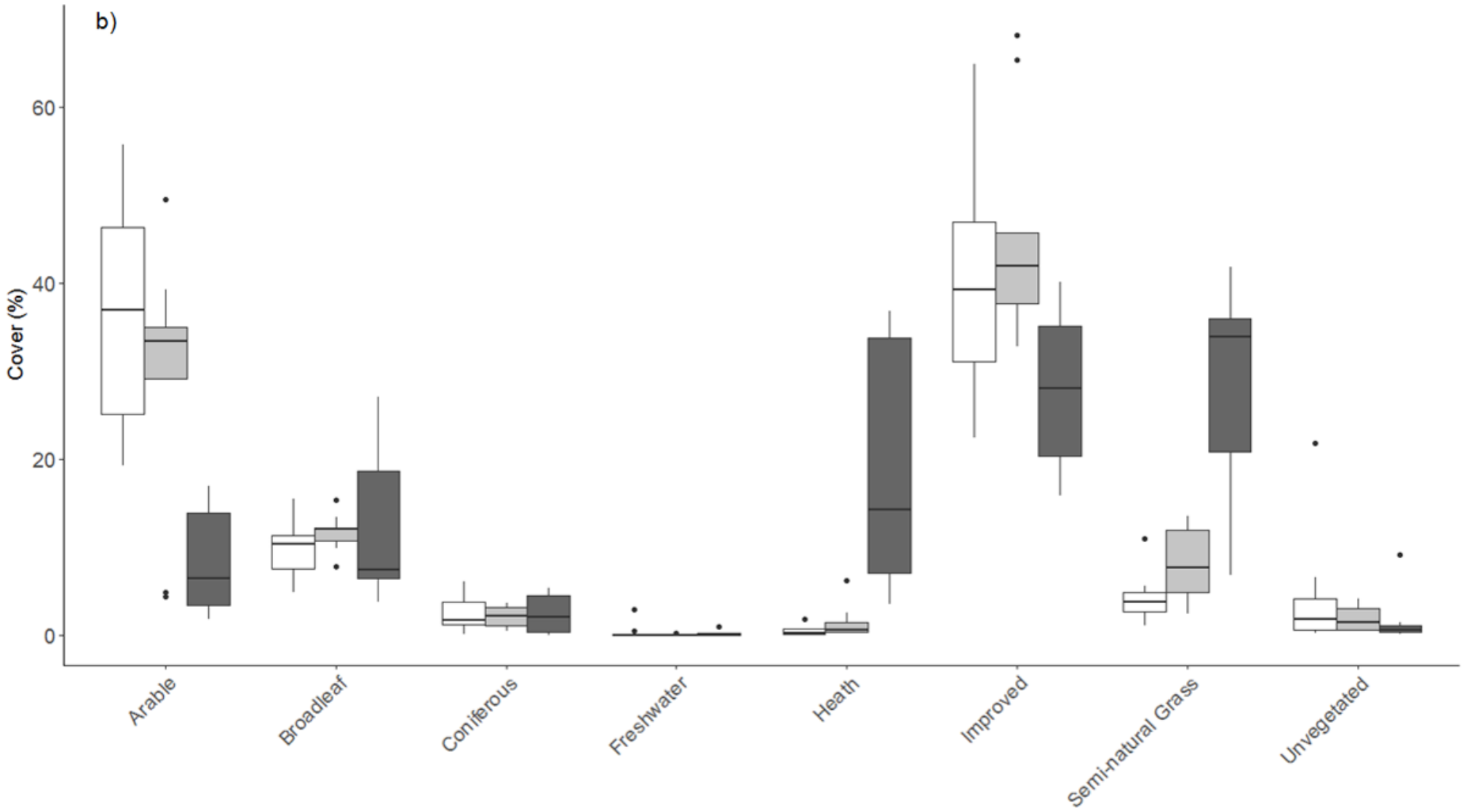
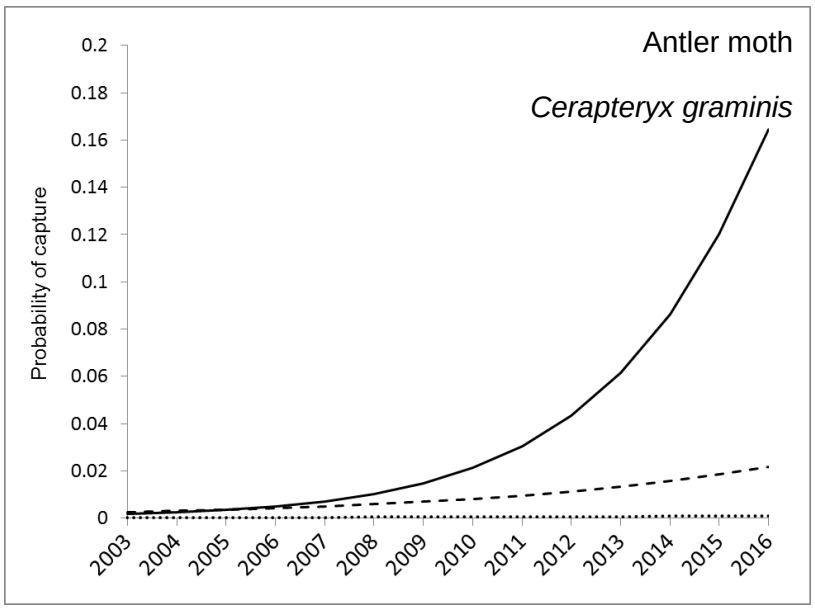
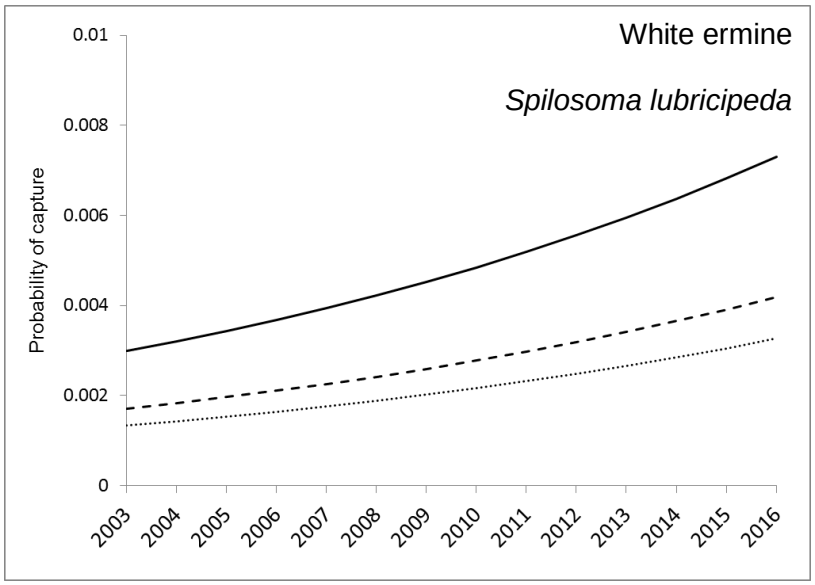
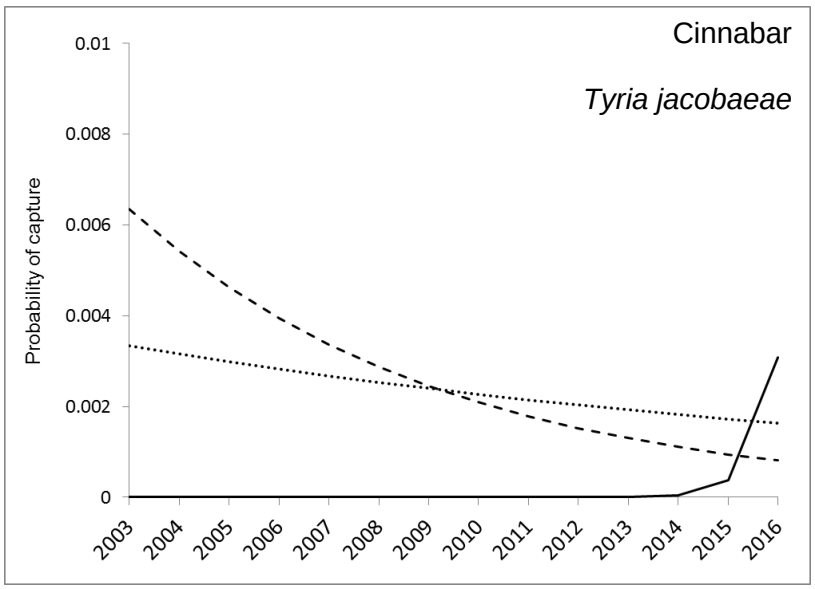
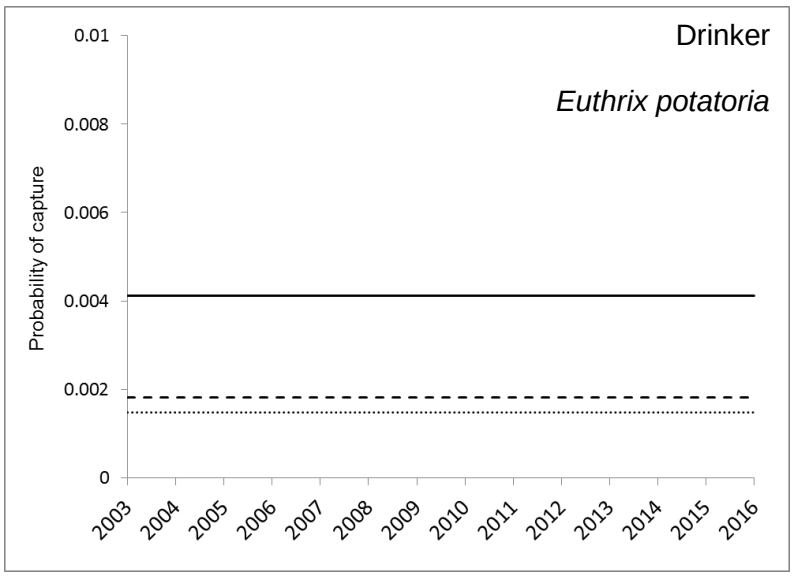


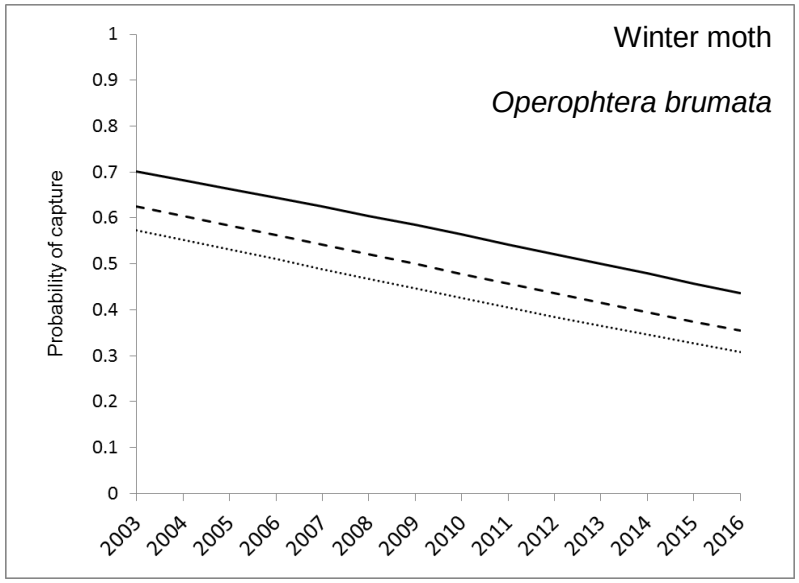
Figure 2. Mean percentage cover of habitat types (see Table 1) in lowland (white, n = 17), buffer (pale grey, n = 9) and core upland (dark grey, n = 8) 10 km squares, calculated across (a) all 1 km grid squares in which moth trapping was carried out (total n = 234: 100 in lowland, 55 in buffer, 79 in core upland) in each 10 km square (matching letters above box plots indicate no significant difference in cover (at $P < 0.05$) in pairwise general linear models) and (b) the entirety of each 10 km square.

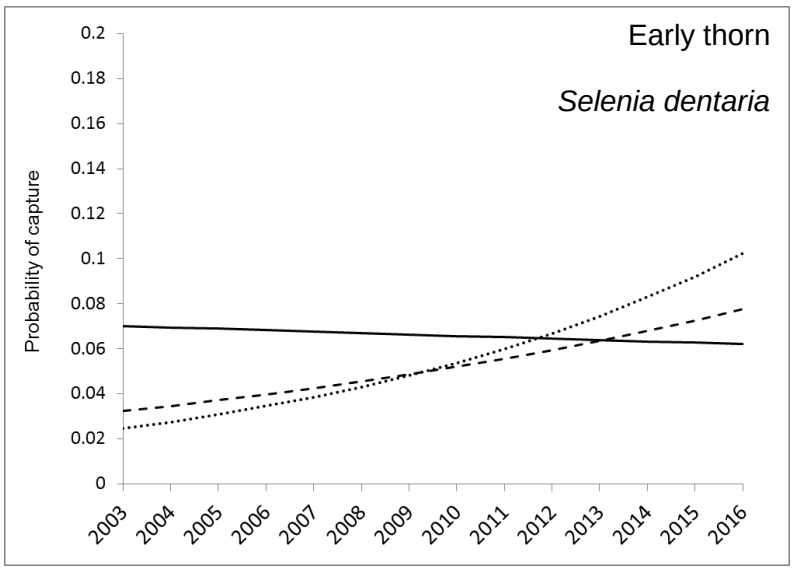

Antler moth
Cerapteryx graminis
Probability of capture
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

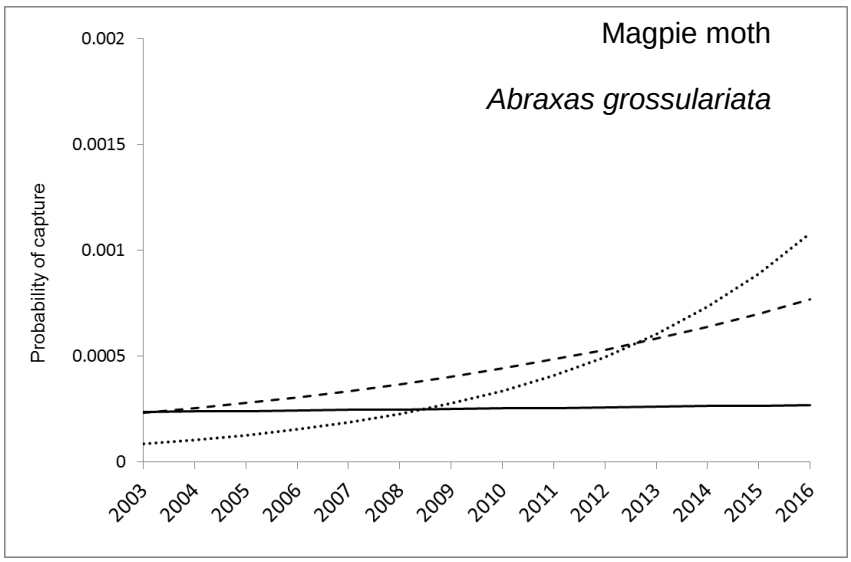

White ermine
Spilosoma lubricipeda
Probability of capture
0.01
0.008
0.006
0.004
0.002
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

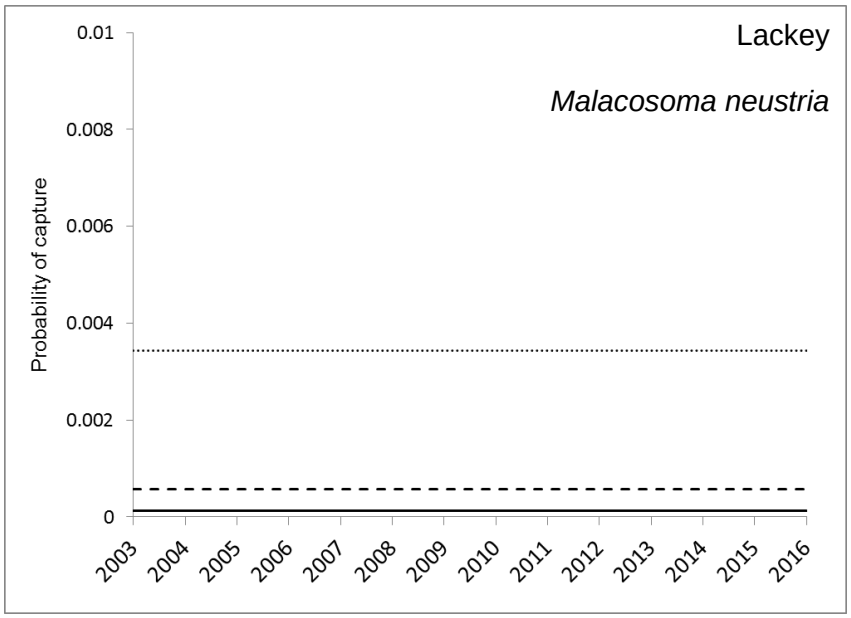

Cinnabar
Tyria jacobaeae
Probability of capture
0.01
0.008
0.006
0.004
0.002
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

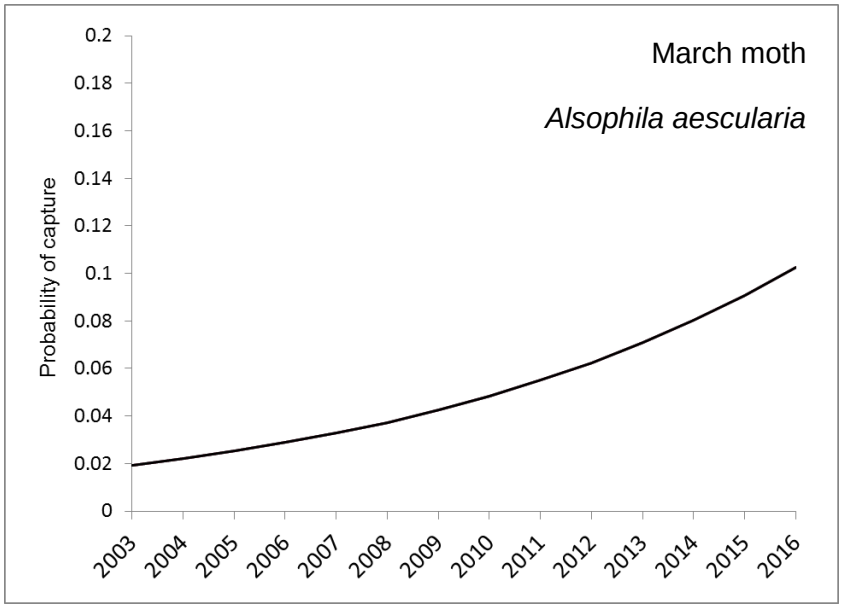

Drinker
Euthrix potatoria
Probability of capture
0.01
0.008
0.006
0.004
0.002
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

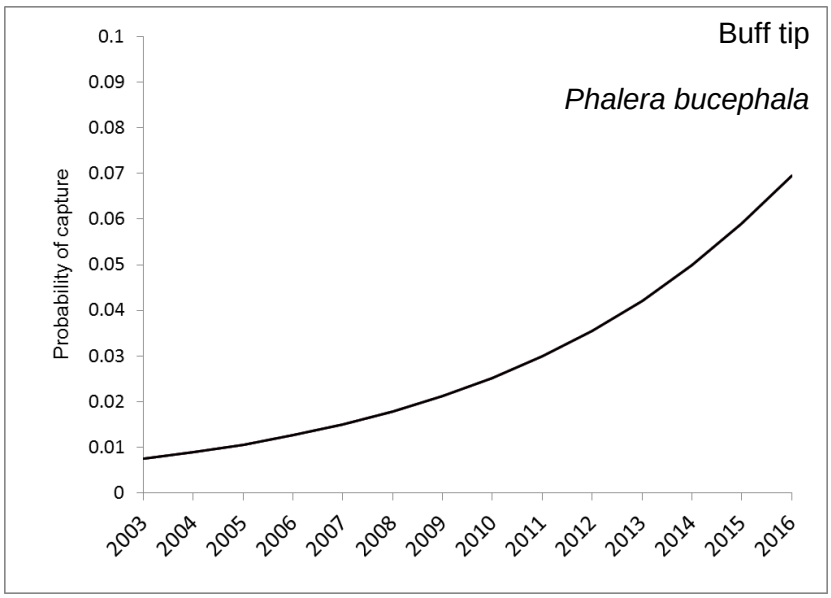

Winter moth
Operophtera brumata
Probability of capture
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

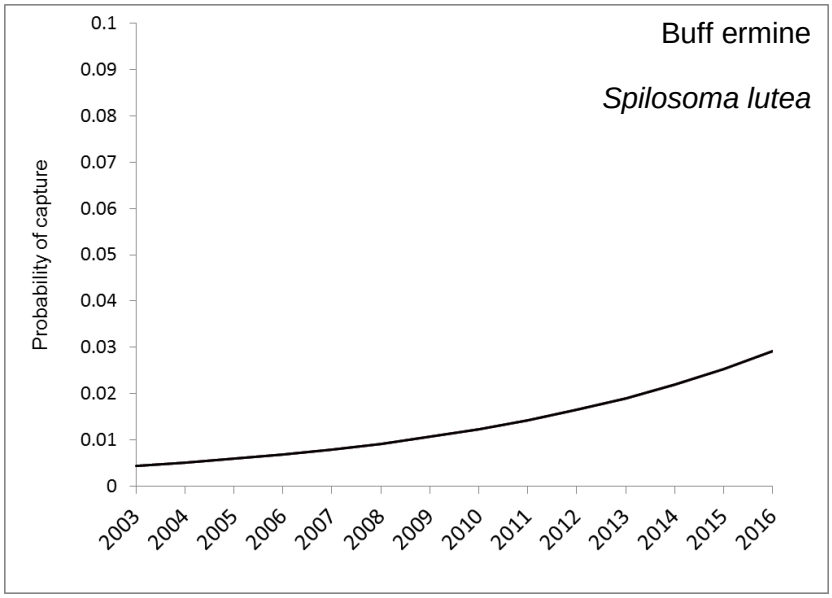

Early thorn
Selenia dentaria
Probability of capture
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

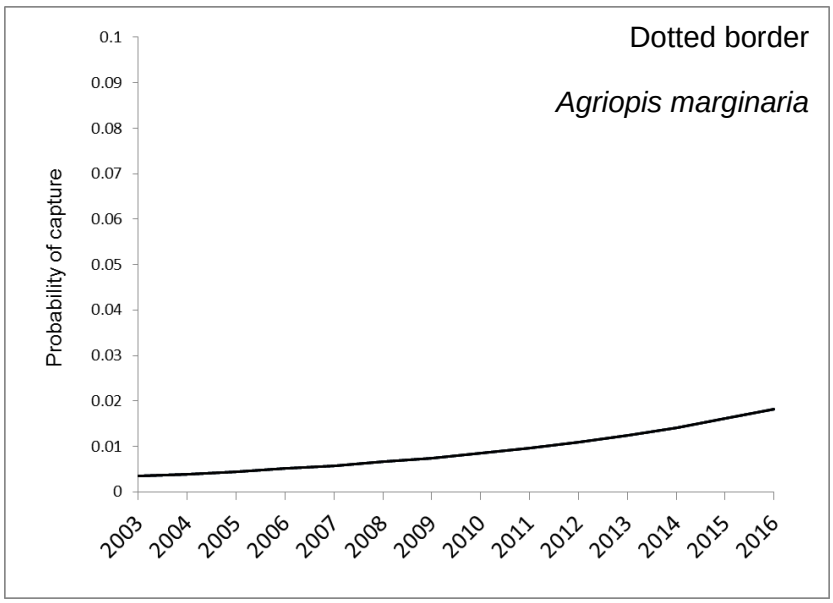

Magpie moth
Abraxas grossulariata
Probability of capture
0.002
0.0015
0.001
0.0005
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

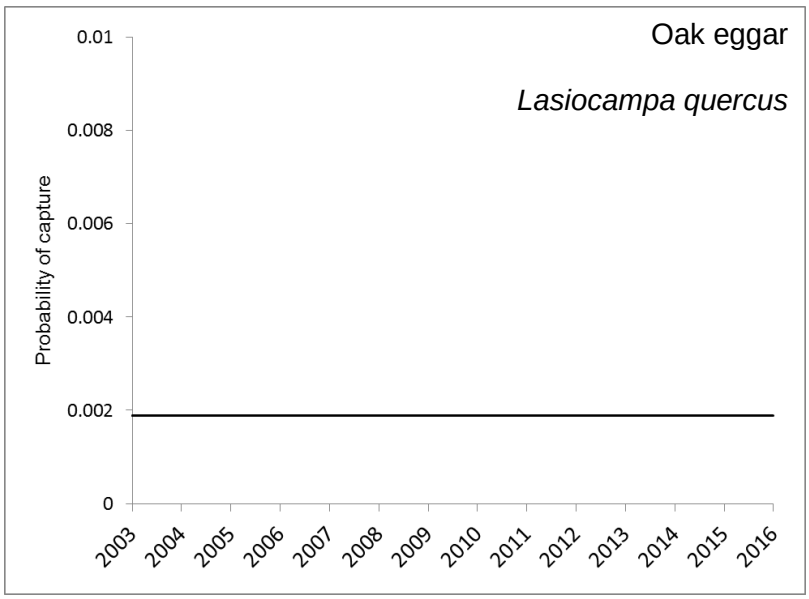

Lackey
Malacosoma neustria
Probability of capture
0.01
0.008
0.006
0.004
0.002
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

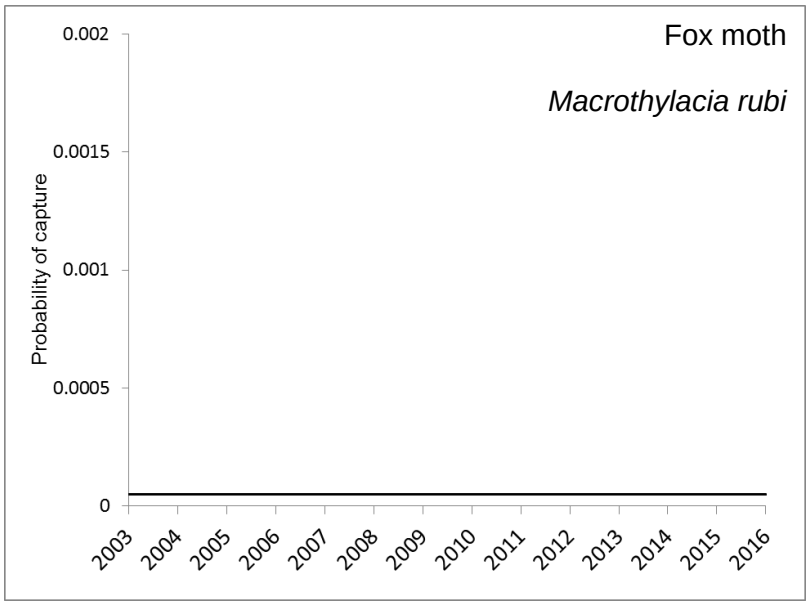

March moth
Alsophila aescularia
Probability of capture
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016


Buff tip
Phalera bucephala
Probability of capture
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016


Buff ermine
Spilosoma lutea
Probability of capture
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016


Dotted border
Agriopis marginaria
Probability of capture
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016


Oak eggar
Lasiocampa quercus
Probability of capture
0.01
0.008
0.006
0.004
0.002
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016


Fox moth
Macrothylacia rubi
Probability of capture
0.002
0.0015
0.001
0.0005
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

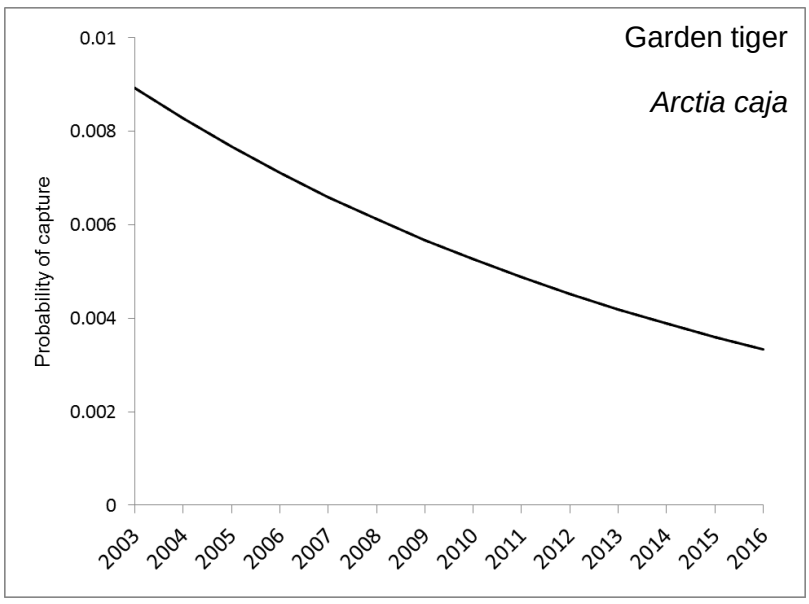

Garden tiger
Arctia caja
Probability of capture
0.01
0.008
0.006
0.004
0.002
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

Figure 3. Modelled capture probability at light traps in Devon, between 2003 and 2016 in lowland (dotted line), buffer (dashed line) and core upland (solid line) squares, for adults of 15 moth species cited to be predated by common cuckoos as larvae (Wyllie 1981). Graphs grouped by similar relationship of occurrence with land use type and year. Plots where a single line is visible indicate no modelled difference in capture probability between lowland, buffer and core upland squares. Model statistics reported in Table 3.

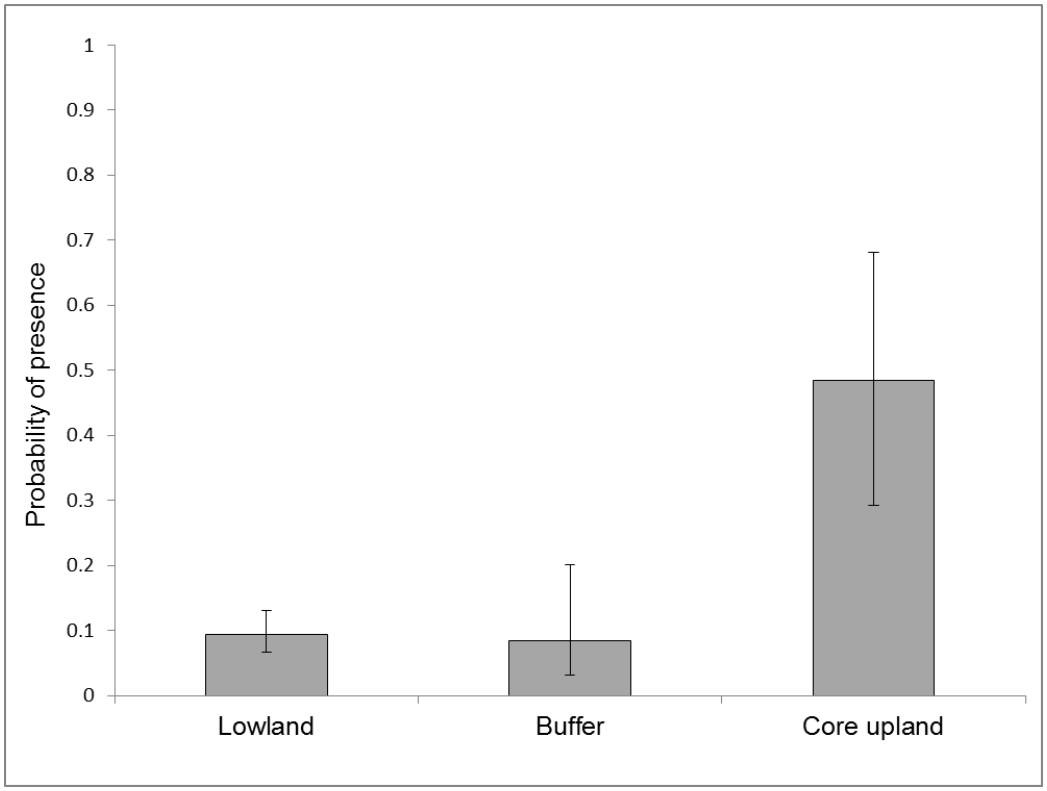


Figure 4. Modelled probability of occurrence of cuckoos in three land use types in Devon from standardised bird atlas surveys. Error bars are standard errors.

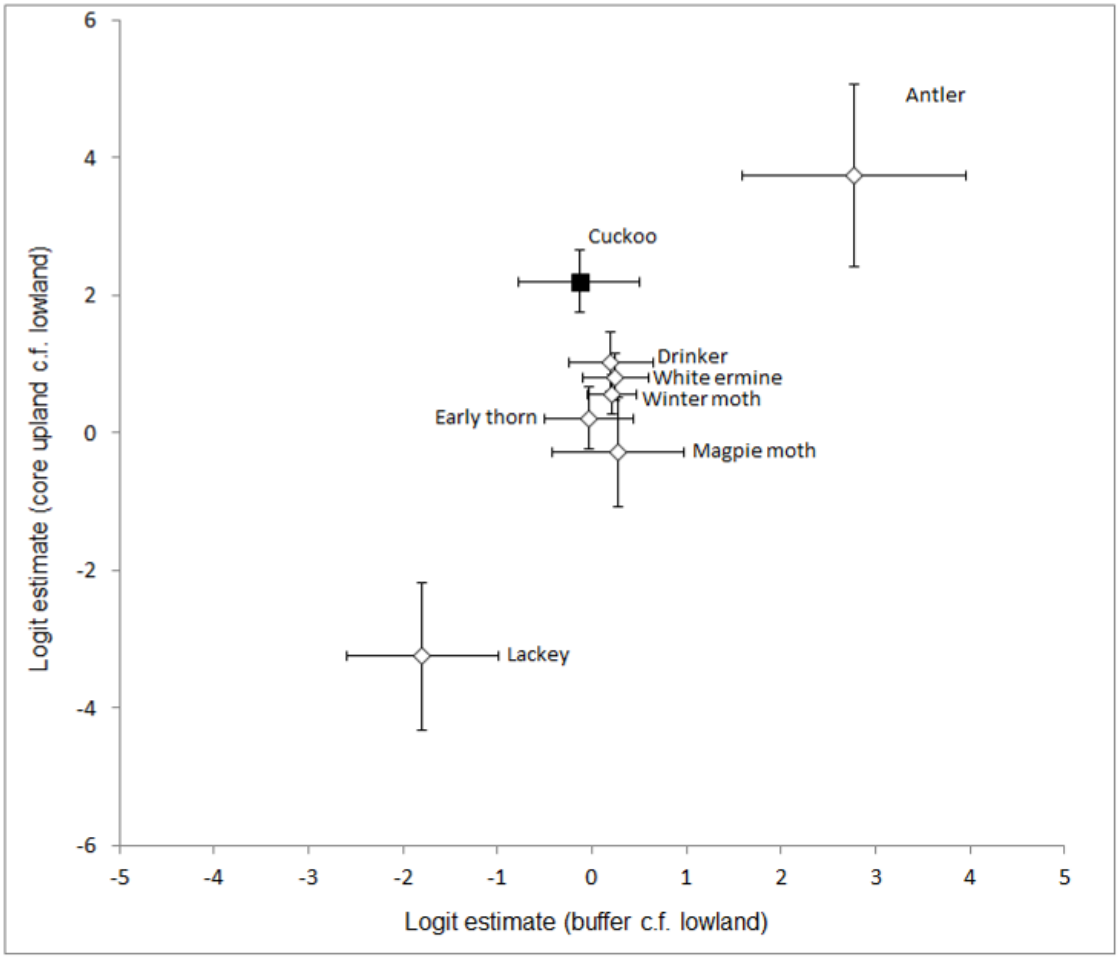


Figure 5. Scatter plot of modelled difference in logit capture probability of moth species (open diamonds) and logit presence probability of cuckoo (filled square) in buffer 10 km squares (x-axis) and core upland squares (y-axis) relative to lowland squares. Error bars show standard errors of estimates (logit). Model statistics reported in Table 3 and in text. For species with a modelled significant interaction term year*square category, estimated effect is calculated using 7th year (midpoint) of time period 2003-16. Species where estimated difference did not significantly differ from zero are not included. Cinnabar not included due to overlarge standard error skewing graph scale.

Table 3. Final selected logistic regression models for variation in logit capture probability in Rothamsted and volunteer light traps in Devon 2003-2016, for adults of 15 moth species cited as cuckoo prey in Wyllie (1981).

Antler moth	Estimate (logit)	S.E.	*P*	
(Intercept)	-8.315	0.988	<0.0001	***
Month = August	1.949	0.165	<0.0001	***
Month = September	0.563	0.212	0.0079	**
Rothamsted c.f. light trap	-8.011	1.240	<0.0001	***
Year	0.106	0.086	0.2165	
Buffer c.f. lowland square	2.347	1.088	0.0310	*
Upland c.f. lowland square	1.907	1.210	0.1149	
ListLength	0.043	0.004	<0.0001	***
Year*Rothamsted trap	0.985	0.117	<0.0001	***
Year*Buffer square	0.060	0.097	0.5357	
Year*Upland square	0.262	0.115	0.0222	*
White ermine				
(Intercept)	-6.618	0.420	<0.0001	***
Month = May	3.121	0.330	<0.0001	***
Month = June	4.022	0.330	<0.0001	***
Month = July	0.692	0.354	0.0504	.
ListLength	0.063	0.003	<0.0001	***
Buffer c.f. lowland square	0.250	0.355	0.4818	
Upland c.f. lowland square	0.810	0.343	0.0184	*
Year	0.069	0.017	<0.0001	***
Cinnabar				
(Intercept)	-5.700	0.788	<0.0001	
Month = June	0.922	0.335	0.0059	
Month = July	-0.712	0.418	0.0887	
ListLength	0.030	0.005	<0.0001	
Buffer c.f. lowland square	0.646	0.988	0.5133	
Upland c.f. lowland square	-27.415	12.832	0.0326	
Year	0.055	0.069	0.4241	
Year*Buffer square	-0.104	0.096	0.2791	
Year*Upland square	2.158	1.012	0.0330	*
Drinker				
(Intercept)	-6.511	0.373	<0.0001	***
Month = July	2.767	0.211	<0.0001	***
Month = August	2.787	0.218	<0.0001	***
ListLength	0.059	0.003	<0.0001	***
Buffer c.f. lowland square	0.201	0.444	0.6513	
Upland c.f. lowland square	1.023	0.432	0.0178	*

Winter moth				
(Intercept)	0.296	0.486	0.5435	
Month = February	-2.736	0.221	<0.0001	***
Month = November	-3.195	0.219	<0.0001	***
Month = December	-0.746	0.165	<0.0001	***
ListLength	0.197	0.028	<0.0001	***
Rothamsted c.f. light trap	-0.790	0.454	0.0819	.
Year	-0.085	0.040	0.0344	*
Buffer c.f. lowland square	0.212	0.262	0.4166	
Upland c.f. lowland square	0.555	0.280	0.0471	*
Year*Rothamsted trap	0.135	0.049	0.0055	**
Early thorn				
(Intercept)	-3.680	0.348	<0.0001	***
Month = April	0.590	0.119	<0.0001	***
Month = May	-1.320	0.142	<0.0001	***
Month = June	-5.292	0.360	<0.0001	***
Month = July	-0.399	0.131	0.0024	**
Month = August	-0.082	0.122	0.5002	
Month = September	-2.387	0.202	<0.0001	***
Rothamsted c.f. light trap	0.959	0.387	0.0132	*
ListLength	0.062	0.003	<0.0001	***
Buffer c.f. lowland square	0.281	0.437	0.5196	
Upland c.f. lowland square	1.096	0.419	0.0088	**
Year	0.116	0.031	0.0002	***
Year*Buffer square	-0.045	0.038	0.2346	
Year*Upland square	-0.126	0.036	0.0004	***
Magpie moth				
(Intercept)	-9.376	0.674	<0.0001	***
Month = July	2.988	0.435	<0.0001	***
Month = August	5.361	0.435	<0.0001	***
Month = September	3.168	0.459	<0.0001	***
ListLength	0.045	0.003	<0.0001	***
Buffer c.f. lowland square	0.996	0.638	0.1188	
Upland c.f. lowland square	1.018	0.730	0.1631	
Year	0.196	0.044	<0.0001	***
Year*Buffer square	-0.103	0.055	0.0599	.
Year*Upland square	-0.186	0.067	0.0056	**
Lackey				
(Intercept)	-5.672	0.592	<0.0001	***
Month = July	1.360	0.306	<0.0001	***
Month = August	-1.074	0.481	0.0255	*
ListLength	0.049	0.005	<0.0001	***
Buffer c.f. lowland square	-1.795	0.803	0.0254	*

Upland c.f. lowland square	-3.253	1.079	0.0026	**
March moth				
(Intercept)	-3.924	0.517	<0.0001	***
March	0.304	0.180	0.0910	.
Month = April	-1.361	0.210	<0.0001	***
ListLength	0.188	0.014	<0.0001	***
Rothamsted c.f. light trap	1.413	0.698	0.0429	*
Year	0.135	0.044	0.0022	**
Year*Rothamsted trap	-0.130	0.053	0.0148	*
Buff tip				
(Intercept)	-4.894	0.306	<0.0001	***
Month = June	0.721	0.129	<0.0001	***
Month = July	0.741	0.132	<0.0001	***
Month = August	-2.511	0.228	<0.0001	***
Rothamsted c.f. light trap	1.858	0.539	0.0006	***
Year	0.177	0.024	<0.0001	***
ListLength	0.051	0.003	<0.0001	***
Year*Rothamsted trap	-0.304	0.039	<0.0001	***
Buff ermine				
(Intercept)	-5.415	0.320	<0.0001	***
Month = June	2.784	0.143	<0.0001	***
Month = July	1.771	0.147	<0.0001	***
Month = August	-0.711	0.179	<0.0001	***
Month = September	-2.728	0.467	<0.0001	***
Rothamsted c.f. light trap	1.717	0.574	0.0028	**
Year	0.147	0.024	<0.0001	***
ListLength	0.062	0.003	<0.0001	***
Year*Rothamsted trap	-0.147	0.032	<0.0001	***
Dotted border				
(Intercept)	-5.661	0.569	<0.0001	***
Month = February	2.540	0.381	<0.0001	***
Month = March	2.716	0.371	<0.0001	***
Month = April	1.010	0.380	0.0079	**
Month = May	-2.423	0.505	<0.0001	***
Rothamsted c.f. light trap	0.850	0.675	0.2084	
Year	0.129	0.038	0.0006	***
ListLength	0.108	0.009	<0.0001	***
Year*Rothamsted trap	-0.119	0.047	0.0109	*
Oak eggar				
(Intercept)	-6.273	0.552	<0.0001	***
Month = August	1.421	0.328	<0.0001	***

ListLength	0.026	0.006	<0.0001	***
Fox moth				
(Intercept)	-9.911	1.586	<0.0001	
ListLength	0.056	0.009	<0.0001	
Rothamsted c.f. light trap	-2.317	1.003	0.0209	*
Garden tiger				
(Intercept)	-4.710	0.465	<0.0001	
Month = July	2.472	0.349	<0.0001	
Month = August	0.826	0.399	0.0385	
ListLength	0.016	0.003	<0.0001	
Rothamsted c.f. light trap	-3.106	0.617	<0.0001	
Year	-0.076	0.033	0.0196	*

Discussion

i) Long-term trends in capture probability of moth species in Rothamsted Insect Survey traps

Garden tiger *A. caja*, cinnabar *T. jacobaeae*, white ermine *S. lubricipeda* and winter moth *O. brumata* occurrence declined in Rothamsted traps that were situated in buffer and core upland 10km squares. In the present study, garden tiger and cinnabar were detected in a survey of cuckoo prey in Britain through analysis of contemporary photographic records. Garden tiger is a formerly highly abundant species that has undergone significant national declines (Conrad *et al.* 2002), and in photographic analysis it was detected only in Scotland where cuckoos are increasing in abundance, particularly in expanses of remaining semi-natural habitat in the Highlands. The garden tiger may therefore feature in the cuckoo diet in parts of its British range where population trends are positive, but there is cause for concern that the analysis here shows a decline in core uplands in Devon.

According to both historic literature and the present study's photographic analysis, cinnabar is preyed upon frequently by juvenile cuckoos between reaching independence and their departure on first migration from Britain. As determined from the literature in an earlier chapter, juvenile cuckoos weigh less than adult birds after fledging from host nests and early in migration. Juveniles therefore dually rely on food sources on the breeding grounds such as cinnabar moths to fuel early migration and again additional body mass. The key larval foodplant of cinnabar is ragwort *Senecio jacobaea*, which is a named species under the UK Weeds Act 1959 and Ragwort Control Act 2003 due to its toxicity to livestock, especially hindgut fermenters such as horses. The legislation places responsibility on landowners to prevent the spread of ragwort "where this presents a high risk of poisoning horses and livestock" (DEFRA 2007). Ragwort declined by roughly 20% in neutral grasslands in Great Britain between 1998 and 2007 (Carey *et al.* 2008), possibly as a result of this legislation. Another common and widespread prey species from photographic analysis, the drinker, displayed a long-term increase in capture probability in Devon.

All Rothamsted traps in the study area were situated in buffer or core upland squares, as opposed to evenly distributed between lowland and core upland (Fig. 1). This means that the traps may be located in areas likely to underestimate declines in the wider landscape, and declines detected by these traps, including key cuckoo prey such as garden tiger and cinnabar, may mask more severe declines in Devon as a whole. While all traps included in this analysis were located within buffer or core upland 10 km squares, the trap site at SX57 was located in a contrastingly exposed site, more remote from woodland and more adjacent to significant human infrastructure than the other included Rothamsted Insect Survey trap sites. This survey site was excluded from several species' analysis having trapped zero individuals over the period in which it was active. However this period was relatively short, and therefore it is not possible to conclude to what extent presence of moths was reduced by the trap's location, surrounding habitat and proximity to infrastructure.

ii) and iii) How does cover of specific habitat types in moth trapping sites vary between broad land management categories? Is variation represented in moth trapping locations?

10 km squares defined as lowland by the present study were distinctive in that arable habitat cover was greater and broadleaf woodland cover was less than buffer or upland squares. Squares defined as upland were distinctive in having most extensive heath and semi-natural grassland, and lowest arable land cover. Squares defined as buffer were distinctive only in intermediate arable land cover (Fig. 2a).

Selection of moth-trap 1km squares made by human observers contributing to the present analysis generally reduced the contrast between land use types compared to differences between entire 10km squares. Differences in habitat cover between the footprint of 1 km squares used for moth trapping and the full 10 km squares suggested siting of moth traps carried bias in favour of broadleaf woodland in all square types, and additionally, bias against arable land in lowland and buffer squares, and against heath and semi-natural grassland and in favour of improved grassland in core upland squares. It is notable that the habitat cover of key upland semi-natural habitats was lower than expected from availability, as these were the habitats for which Dartmoor is primarily designated as a National Park, and the park was the focus of 10 km square selection for upland semi-natural areas in the present study. Differences in moth occurrence between land use types in subsequent analyses therefore may be responses to a smaller difference in habitat cover than is actually recorded at the full 10km square scale.

Some of the selection of moth trapping locations is likely to relate to observers attempting to maximise proximity of both open and enclosed habitats and therefore ability to trap a greater proportion of local moth diversity. Moth trapping success is also negatively affected by greater wind speed and exposure (New 2004), so that wooded habitats may be selected for the shelter they provide from wind. The size, weight and power requirements of MV light traps limit their suitability to remote locations (New 2004).

iv) How does adult capture probability for moth species vary between land management categories in Devon, and do temporal trends in adult abundance differ?

In models for several moth species, difference in capture probability relative to lowland squares was statistically significant for core upland but not for buffer squares (Table 3). From analysis of differences in habitat cover between land management categories (Fig. 2a) the habitat variables which also displayed this relationship with land management category were heath and semi-natural habitat. Among species showing significantly higher capture probability in core upland squares relative to buffer and lowland squares, drinker and white ermine are associated with seminatural grass moor and heath (Waring & Townsend 2017). Drinker moth and oak eggar larvae are particularly widespread and common prey species of the cuckoo, including within the photographic analysis of prey across Britain that covered a highly similar period to this analysis of captures. Drinker foodplants are coarse grasses and reeds likely to be outcompeted and grazed out of improved pastures by reseeding and high grazing stocks. Drinker moth is therefore likely to show greater occurrence on Dartmoor due to greater diversity of grasses in the predominantly semi-natural grassland than in improved pastures that predominate in lowlands. Oak eggar foodplants include heath in uplands but also eat a variety of woody plant species. Lack of difference in modelled capture probability between land use types, unlike for drinker, may relate to this broad range of food plants. However captures of this species were relatively rare and predictive power of models to model occurrence responses to land management type may have been reduced.

Capture probabilities of lackey and antler moth were significantly lower and higher, respectively, in buffer and core upland relative to lowland (Table 3) (Fig. 5). Antler moth capture probability may be higher in buffer and core squares due to increased broadleaf woodland cover and lower arable land cover. Antler moth preference for grass or heath moor habitats, and grass species that dominate seminatural grassland (e.g. *Festuca ovina*, *Molinia caerulea*) as larval foodplants (Waring & Townsend 2017), suggest that arable land is of poor suitability for this species.

Lackey habitat preference is for open habitat but larval foodplants are trees and shrubs that commonly occur in hedgerow field boundaries and woodland stands in arable land (hawthorn, blackthorn, oak, willow), which may explain why lackey capture probability is higher in lowlands. Land use intensification has increased mechanical cutting and flailing of boundary hedgerows, but this practice is rare in April to June when larvae are aggregating on food plants.

Garden tiger and winter moth declined in all land use types. The trend for garden tiger reflects national trends for this formerly common and widespread species (Conrad *et al.* 2002), but the fact that rate of decline was no less severe in core uplands may still be cause for concern. Winter moth occurrence was higher in core uplands but showed similar modelled rate of decline. As well as being a reported cuckoo prey species, it is a key larval food source for both resident and migrant woodland passerines, and upland woodlands are considered a stronghold in Devon for some of these species such as pied flycatcher *Ficedula hypoleuca*. A similar modelled rate of winter moth decline in uplands is a sign of potential widespread decline in this species in Devon. Such declines may relate to climate rather than land use effects (Fox *et al.* 2014). Cinnabar moths showed decline in lowland and buffer squares but increases in core upland. It is possible this represents the species increasingly retreating to uplands where control of the larval foodplant ragwort is less intensive, though the plant is still likely to be confined mainly to enclosed grasslands as opposed to open moor.

Information on design of small light traps used, including bulb type (actinic, mercury vapour) and bulb power, were available from very few sites, therefore variation in the use of different trap types could not be directly accounted for within the analysis. However trap type is likely to have a significant effect on numbers and taxa of moth captured (Merckx & Slade 2014). The study attempted to mitigate effects of changes in trap used by including the interaction term between year and broad trapping method. The effect was significant in models for a number of species in that capture probability trends were more positive in small light traps. This suggests that improvements in light traps may have underestimated declines relative to the more standardised Rothamsted trap method.

v) Do cuckoos show different rate of occurrence between land-management types?

Cuckoos had higher rate of occurrence in core uplands. This is expected to relate to presence of nesting populations of hosts such as meadow pipits, as well as prey availability. Cuckoo prey on Dartmoor was shown in the present study to feature drinker moth, grasshoppers (Orthoptera) and flies such as Rhagionidae and Tipulidae that are likely to benefit from the core uplands' increased presence of semi-natural grassland, and decreased presence of arable land practices such as pesticide use. Difference in cuckoo presence/absence between land management categories was most similar to those modelled in moth trapping analyses for drinker (Fig. 5). This was the most frequent prey species detected in a previous analysis of adult cuckoo faecal samples on Dartmoor (Chapter 5).

Cuckoos typically consume larval prey, and abundance of trapped adult Lepidoptera may not readily reflect abundance of larvae. Long term changes in adult population may see an opposite trend in fecundity or general productivity, especially if there is a significant density-dependent effect on fecundity, or availability of food plant or egg-laying substrate as indicated for cinnabar (Dempster & Lakhani 1979).

Conclusions

Analysis of Rothamsted Insect Survey data has shown that the long-term variation in occurrence of 'cuckoo prey' moth species in standardised trapping is variable. However, adult cinnabar and garden tiger moths, shown elsewhere in this study to remain key cuckoo prey for juvenile and adult, respectively, have shown significant declines since the 1970s and this may have contributed to the significant cuckoo declines in this period. Critically, neither of these species is strongly associated with upland semi-natural grassland and heathland. Over a shorter period where land use could also be modelled, drinker and oak eggar which are key adult cuckoo prey as larvae remained stable in rate of occurrence. Both are large lasiocampids of semi-natural habitats. Drinker had

higher occurrence in core uplands and showed most similar relationship between occurrence and land use to the cuckoo. Cinnabar moths also increased in core uplands. Stability and increases in key prey moths in the uplands may explain why cuckoo breeding abundance trend is positive in vicinity of Dartmoor. A key issue of reporting-rate bases for trend estimates for very abundant species is that abundance is capable of significantly decreasing while presence on species lists shows no significant change, and reduction in presence may ultimately only begin to be captured during stages of potentially rapid disappearance from survey sites (Kamp *et al.* 2016). The implications of this to the present study is that species appearing stable may in fact be in decline without loss of occurrence rate, therefore analysis from the fewer sites for which abundance data in traps is available would be a critical next step in establishing health of cuckoo prey populations.

References

Balmer, D., Gillings, S., Caffrey, B., Swann, B., Downie, I., and Fuller, F. (2013) *Bird Atlas 2007–11.* Thetford: BTO.

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67(1): 1–48.

Beavan, S. D., and Lock, M. eds. (2016) *Devon Bird Atlas: 2007-2013*. Devon Birds.

Carey, P. D., Wallis, S., Chamberlain, P. M., Cooper, A., Emmett, B. A., Maskell, L. C., McCann, T., Murphy, J., Norton, L. R., Reynolds, B., Scott, W. A., Simpson, I. C., Smart, S. M., and Ullyett, J. M. (2008) *UK Results from 2007 Countryside Survey*. (June 2009.). Centre for Ecology & Hydrology.

Conrad, K. F., Warren, M. S., Fox, R., Parsons, M. S., and Woiwod, I. P. (2006) Rapid declines of common, widespread British moths provide evidence of an insect biodiversity crisis. *Biological Conservation* 132(3): 279–291.

Defra (2007) *Code of Practice on How to Prevent the Spread of Ragwort - PB9840 revised March 2007*. London.

Dempster, J. P., and Lakhani, K. H. (1979). A population model for cinnabar moth and its food plant, ragwort. *The Journal of Animal Ecology*, 143-163.

Denerley, C. (2014) *The impact of land use change on a brood parasite system: cuckoos, their hosts and prey*. University of Aberdeen.

Denerley, C., Redpath, S. M., van der Wal, R., Newson, S. E., Chapman, J. W., and Wilson, J. D. (2018) Breeding ground correlates of the distribution and decline of the Common Cuckoo *Cuculus canorus* at two spatial scales. *Ibis*. doi:10.1111/ibi.12612.

Grant, B. S., Owen, D. F., and Clarke, C. A. (1996) Parallel Rise and Fall of Melanic Peppered Moths in America and Britain. *Journal of Heredity* 87: 351–357.

Harris, S. J., Massimino, D., Gillings, S., Eaton, M. A., Noble, D. G., Balmer, D. E., Procter, D., and Pearce-Higgins, J.W. Woodcock, P. (2018) *The Breeding Bird Survey 2017. BTO Research Report 706*. Thetford.

Hewson, C. M., Thorup, K., Pearce-Higgins, J. W., and Atkinson, P. W. (2016) Population decline is linked to migration route in the Common Cuckoo. *Nature Communications* 7: 12296.

Intachat, J. and Woiwod, I.P. (1999). Trap design for monitoring moth biodiversity in tropical rainforests. *Bull. Entomol. Res*. 89: 153–163.

Kamp, J., Oppel, S., Heldbjerg, H., Nyegaard, T., and Donald, P. F. (2016) Unstructured citizen science data fail to detect long-term population declines of common birds in Denmark. (Schröder, B., Ed.) *Diversity and Distributions* 22(10): 1024–1035.

Mangels, J., Fiedler, K., Schneider, F. D., and Blüthgen, N. (2017) Diversity and trait composition of moths respond to land-use intensification in grasslands: generalists replace specialists. *Biodiversity and Conservation* 26(14): 3385–3405.

Massimino, D., Woodward, I.D., Hammond, M.J., Harris, S.J., Leech, D.I., Noble, D.G., Walker, R.H., Barimore, C., Dadam, D., Eglington, S.M., Marchant,

J.H., Sullivan, M.J.P., Baillie, S.R. and Robinson, R. A. (2017) *BirdTrends 2017: trends in numbers, breeding success and survival for UK breeding birds. Research Report 704.* Thetford.

Merckx, T. and Slade, E.M., (2014). Macro-moth families differ in their attraction to light: implications for light-trap monitoring programmes. *Insect Conservation and Diversity*, *7*(5): 453-461.

Morton, D., Rowland, C., Wood, C., Meek, L., Marston, C., Smith, G., Wadsworth, R., and Simpson, I. C. (2011) *Final Report for LCM2007 – the new UK Land Cover Map. CS Technical Report No 11/07.*

New, T. R. (2004) Moths (Insecta : Lepidoptera) and conservation: background and perspective. *Journal of Insect Conservation* 8: 79–94.

PECBMS (/2019) Pan-European Common Bird Monitoring Scheme. Accessed: <https://pecbms.info/. >.

Roberts, R. L., Donald, P. F., and Green, R. E. (2007) Using simple species lists to monitor trends in animal populations: new methods and a comparison with independent data. *Animal Conservation* 10(3): 332–339.

Waring, P., and Townsend, M. (2017) *Field guide to the moths of Great Britain and Ireland*. Bloomsbury Publishing.

Willemoes, M., Strandberg, R., Klaassen, R. H. G., Tøttrup, A. P., Vardanis, Y., Howey, P. W., Thorup, K., Wikelski, M., and Alerstam, T. (2014) Narrow-front loop migration in a population of the common cuckoo *Cuculus canorus*, as revealed by satellite telemetry. *PloS one* 9(1): e83515.

Williams C.B., French R.A. and Hosni M.M. (1955). A second experiment on testing the relative efficiency of insect traps. *Bull. Entomol. Res.* 46(93–204): 46.

Wyllie, I. (1981) *The Cuckoo*. London: Batsford.

7. General discussion and conclusions

Introduction

The common cuckoo *Cuculus canorus* has undergone severe declines in many European countries across its Palaearctic breeding range (PECBMS 2019). In Britain, significant variation in breeding population trend between habitats suggests declines may relate to land use. This entails relatively positive trends in landscapes with greater semi-natural habitat cover, and lower influence of intensive land use types such as arable agriculture (Massimino *et al.* 2017, Denerley *et al.* 2018).

The focus of the present study was to identify key habitat and prey resources of the cuckoo and its main host species the meadow pipit *Anthus pratensis* in upland semi-natural grassland (a relative stronghold habitat for the cuckoo in Britain) and determine how these resources may be affected by past, current and future land-use in Britain. In chapter 1, synthesis of the literature on the cuckoo and its hosts highlighted mechanisms through which the cuckoo's lifestyle as a brood parasite and long-distance migrant, with an exclusively invertebrate diet, increased its vulnerability to environmental change on breeding grounds. This included evidence of individual specialism in breeding habitat, which had implications for sensitivity to habitat loss and population viability through reduced mate availability. Two data chapters focused on the nestling cuckoo's habitat and food resources as possible factors through which environmental impacts may act on the demographic factor of cuckoo nestling survival. In chapter 3, unparasitised meadow pipits were shown to select foraging habitat with structural heterogeneity in upland semi-natural grasslands, including edge habitats, but preferentially foraged on patches of uniformly short grasses. In chapter 4, foraging habitat selection and nestling-provisioning rate of meadow pipit pairs did not significantly differ between those raising a cuckoo and those raising a pipit brood, but volume of provisioned food per hour and foraging distances were both higher when provisioning fledgling cuckoos than provisioning nestlings of either cuckoo or meadow pipit. DNA sequencing

approaches to nestling diet study were applied for the first time to the common cuckoo, and showed nestling diet differed between cuckoos and hosts. Two data chapters focused on the adult cuckoo's food resource as a factor through which environmental factors may readily act on the demographic factors of adult survival and fecundity. In chapter 5, DNA sequencing was applied for the first time to the study of diet of adult cuckoos, and images from photographic social media were used as a novel survey tool to identify lepidopteran prey of the cuckoo in Britain. Further to both analyses showing that lasiocampid moths are frequent prey, DNA sequencing highlighted unexpected high occurrence of Orthoptera and Diptera families such as Rhagionidae. In chapter 6, the temporal and land use trends in occurrence of 'cuckoo prey' moth species showed long term declines in three key species captured in the photographic analysis in chapter 5. Two key Dartmoor prey species from DNA analysis in chapter 5 showed temporally stable rates of occurrence, and pattern of occurrence of cuckoos between land use types most closely resembled that of the most frequent Dartmoor prey species.

Here, I analyse a selection of the study's key findings regarding their implications for how land use influences common cuckoo populations, and their significance to determining future research and conservation action. I have focused on findings relating to meadow pipit habitat preference, as the key host in a cuckoo stronghold habitat within England and one of two common hosts in Britain; and findings from assessing the cuckoo nestling and fledgling resource requirements in this habitat that potentially distinguish early-stage cuckoo ecology from that of its host. Focus is also given to the successful application of DNA sequencing and novel surveys of images in online photographic platforms, which provided the first detailed analysis of cuckoo diet since the onset of long-term decline in 1985; and DNA-based methods offered a potential means of overcoming biases of largely field observation-based knowledge of the diet. Relationship of the key prey taxa to land management practice is examined due to their potential vulnerability to land use intensification.

Analysis of key findings of the present study and messages for future research and conservation

Host meadow pipit foraging habitat preference

The multi-scale analysis of habitat use in the present study suggested that meadow pipits, the key cuckoo host in upland semi-natural grassland, preferred foraging habitat heterogeneity at the 50 x 50 m scale in semi-natural grasslands, with preference for partial cover of tall semi-natural grassland grasses (*Festuca ovina*, *Agrostis sp.*) and gorse *Ulex* shrubs, and presence of trees; but preference for 10 x 10 m foraging plots within these areas of grasses that have short and uniform sward (< 10 cm) typically caused by animal grazing and trampling. Meadow pipits also selected 50 x 50 m squares that contained enclosed improved grassland of similarly short uniform sward height due to grazing. Selection and avoidance of vegetation types on 10 x 10 m foraging plots were similar when host pairs were raising a cuckoo nestling rather than their own young, suggesting that raising a cuckoo nestling had no marked influence on foraging habitat selection. These findings cumulatively indicate that **breeding by meadow pipits and cuckoos in semi-natural grasslands benefits from variable vegetation complexity, from short grass to shrub and tree growth**. This has significant implications for meadow pipits' relationship with land management in upland semi-natural habitats. Open semi-natural habitats originate from **t**he clearance of woodland by earlier human hunting and agriculture, to create areas that were more readily hunted and grazed, respectively. Within upland semi-natural landscapes there is scope for a wide diversity of habitat structures (primarily relating to extent of shrub and tree vegetation) to co-exist; from treeless, montane-like areas of dwarf-shrubs to stands of ancient oak woodland. There is much debate regarding the extent to which this diversity of habitats is realised in British uplands by stakeholders in their land management (Monbiot 2016, Harrison 2016). Debate is often centred on the relative influence wielded by grazing interests, and additionally in the north of Britain, driven grouse shooting. Upland semi-natural habitats are predominantly of acidic soil unsuitable for arable crop production. Land uses for generation of revenue are more commonly livestock grazing, forestry (with mostly non-native conifers such as spruce *Picea*) for timber production

(Spencer & Haworth 2005), and in heathland, maintenance of red grouse *Lagopus lagopus scotica* populations for driven shooting.

Afforestation of grassland and heathland eventually eliminates open habitats and similarly to agricultural improvement reduces heterogeneity of low vegetation. Meadow pipit numbers actually increase in plantations shortly after establishment (Moss *et al.* 1979) but rapidly decline as growth progresses (Lack 1933). More recently tree and forest removal has taken place in heathlands and even seedling trees may be removed as part of upland bog conservation or restoration (Scottish Government 2016). However, forestry areas are still maintained in acidic soil areas in Britain, in both lowlands and uplands, according to Land Cover statistics derived from LCM2015 (Rowland *et al.* 2017). Proposals for new tree planting or tree preservation measures in grazed areas are often met with controversy among upland stakeholders, even with native species (e.g. North York Moors National Park Authority 2012).

Livestock grazing in upland grasslands may be preceded by improvement practices including reseeding with competitive grasses (such as perennial ryegrass *Lolium perenne*), fertilizer application, and drainage which together reduce herb vegetation diversity (Fuller 1987). This has been the fate of much lowland semi-natural grassland not suitable for conversion to arable land (Fuller 1987). Critically, grazing with high densities of livestock is likely to reduce and suppress height and diversity of most vegetation in both enclosed and unenclosed grasslands and heathlands, and maintenance of grouse moors often includes removal of all trees as they are potential nest sites for carrion crows *Corvus corone* that predate grouse chicks. Both upland grazing and grouse moor management include burning of areas where growth of gorse or heather is considered to have advanced beyond the optimum growth for grazing animals or grouse, respectively.

Besides afforestation, which results in too high a density of trees for meadow pipit breeding, the drive to maintain financial viability of infertile acidic soil areas in uplands generally steers land management towards widespread reduction in vegetation height, complexity and heterogeneity, including tree and scrub removal. However, the present study suggests that management of upland

grasslands and heathlands that removes tall herb, shrub and tree vegetation, or hinders their regeneration, is likely to be detrimental to both meadow pipits and the cuckoos that parasitise them. At the same time, upland heath and grassland's suitability for meadow pipits and cuckoo parasitism depends on anthropogenic management, most notably an appropriate grazing intensity. Grazing previously shown to be associated with stable or increasing breeding meadow pipit abundance was below a density of 1.0 ewes ha^{-1} under experimental conditions in *Molinia* dominated uplands (Evans *et al.* 2006). Grazing density in the present study area was dynamic due to the presence of multiple grazer species under the control of several commoners' association members, and thus difficult to quantify.

At the present study area on Dartmoor, meadow pipits and cuckoos made use of habitats adjacent to semi-natural grasslands to forage, these being enclosed improved pastoral grasslands and woodland, respectively. This further supports that the cuckoo and meadow pipit benefit from landscape-scale heterogeneity including the presence of final woodland succession. The finding that meadow pipits forage in the vicinity of trees and edge habitat is additionally significant as it demonstrates similarity in habitat preference to cuckoos in the study area. The study area was selected due to presence of both meadow pipits and cuckoos, and trees were present (although unevenly distributed) across most of the study area. However, meadow pipit foraging was significantly positively associated with tree presence. Elsewhere in the present study, cuckoos fledged from meadow pipit nests moved gradually to edges of woodland, sometimes more than 300 m from the nest, while still receiving provisioning from meadow pipits. During faecal sample collection from perching adult cuckoos in the field, trees were in continuous use during male song and display, female host-nest watching, and foraging on prey from low vegetation (pers. obs.).

Meadow pipits hosting a cuckoo provided a different nestling diet than those feeding their own brood

Observation of provisioning visit rates, prey load size, foraging distances and habitat use of meadow pipits raising their own young or cuckoo young indicated

that nestling cuckoos were delivered a similar volume of prey per hour as host broods. There was no significant difference in provisioning visit rate or prey load size between nestling cuckoos and meadow pipit broods, and from additionally measuring foraging distances and habitat use it was concluded that host pairs did not compensate when provisioning a cuckoo nestling, by selecting different foraging habitat or flying different distances to forage. However, when observation sessions of hosts feeding fledgling cuckoos were included, this indicated that provisioning visit rates to cuckoos were significantly more frequent during the fledgling stage, and foraging distances were significantly greater, than during the cuckoo or pipit nestling stage. There was no detected difference in prey load size provisioned to cuckoo fledglings versus nestlings or meadow pipit broods, indicating that cuckoo fledglings were fed a higher volume of prey per unit time than nestling cuckoos or pipit broods. Higher rates of provisioning visits coupled with greater foraging distances indicated there was an increase in host-parental energy investment per foraging trip during the cuckoo fledgling stage relative to the nestling stage. The duration of the cuckoo nestling and fledgling stage is additionally longer than that of hosts, reported by Wyllie (1981) and also noted in this study, therefore absolute energy investment in raising a cuckoo also exceeded that for raising a meadow pipit brood. Analysis of prey DNA in faeces from meadow pipit broods and cuckoo nestlings and fledglings, indicated that young cuckoos were fed a broader diversity of prey, and were fed Diptera of all sizes with significantly greater frequency than were pipit broods. This suggested that meadow pipits provision cuckoos with different prey to conspecific broods. As foraging habitat preference did not differ between pairs raising pipit broods and cuckoos, this difference (and the higher diversity of prey in cuckoos) indicated hosts have different selectivity when foraging to provision a cuckoo. Greater prey diversity suggests reduced prey selectivity in parasitised host pairs, as was suggested occurred in reed warblers provisioning cuckoo nestlings at advanced stages (Grim & Honza 2001). However it is ultimately not possible to determine whether the diet difference when provisioning a cuckoo signified lower selectivity, or redirected selectivity given the larger size of the nestling.

Returning to hypotheses tested in the present study, critically the results have indicated that **raising a cuckoo nestling to independence in semi-natural grassland has different resource requirements to raising a host brood**. Host pairs invested greater absolute energy raising a cuckoo, and food resources differed between parasitised and unparasitised nests. The immediate implication of this is that equivalent resource availability to meadow pipits may result in lower cuckoo fledgling success than that of hosts. Daily nest failure rates were compared between parasitised and unparasitised nests, and failure rate was higher for parasitised nests, but this did not relate to provisioning rate and the main cause of cuckoo nestling-stage failure was predation. It has been suggestedthat begging calls made by the cuckoo to encourage provisioning are limited by predation risk (Brooke & Davies 1989), but without data on feeding state or begging call volume it is not possible to distinguish causes of predation and determine whether nest failure by predation linked to insufficient food resource in the cuckoo. To determine the potential for different resource requirement of cuckoos to influence nest failure rate, daily nest survival of parasitised and unparasitised nests would need to be measured under reduced (or absence of) predation risk such as within a predator exclusion or control regime.

The analysis in the present study had limitations relating primarily to the observational as opposed to experimental approach to studying provisioning and diet. Pairs raising cuckoo nestlings were all naturally parasitised by cuckoos, with no cross-fostering carried out (as was conducted by Kleven *et al.* 1999 and Martín-Gálvez *et al.* 2005) to allow observation of the same pairs in turn provisioning a host brood or a cuckoo nestling. This technique would assist in ruling out effect of host pair quality on provisioning behaviour. This is potentially important as pairs with certain characteristics may be selected by cuckoos, such as being easier to parasitise due to poor nest attendance, or potentially higher quality foster parents using physical or behavioural cues earlier in the season. Determining the existence and nature of this selectivity would be a considerable challenge, but cross fostering could eliminate such an effect from provisioning analyses. Due to the potential vulnerability of ground nests to over-attendance by human observers, compared to the relative

protection of cross fostering to elevated nests as in Brooke and Davies' study (1980) it was not considered appropriate to cross-foster young in the present study. The reliance on observing naturally parasitised pipit pairs also limited the sample size of parasitised nests. These nests received additional observation sessions and nest identity was accounted for as a random effect in all analyses, but limited parasitised nests is likely to have limited the predictive power of some models to detect effects of nestling species on response variables.

Fledgling cuckoos could receive observation sessions identically to nests due to their characteristics of being readily detectable on successive days from their call, and remaining in single locations for long periods while receiving host provisioning. However fledgling meadow pipits were not observed in this way due to the presence of multiple, mobile birds that often could not be detected. It was was therefore not possible to also quantify the provisioning rates, prey load size, foraging distances or prey of pipit pairs with host fledglings, and capture whether any of these increased post-fledging as with the cuckoo.

Aspects of cuckoo ecology, especially nestling ecology, show variability between host species. This has the capacity to influence cuckoo demographic factors such as nestling and juvenile survival, and therefore populations. Further study is therefore required comparing provisioning, nestling diet and foraging distance and habitat use between cuckoo nestlings and host broods in additional habitats and host species. The priority species for this research in Britain is the reed warbler *Acrocephalus scirpaceus* as the other common host in this region (Brooke & Davies 1987), but for addressing further questions of how land-use type influences cuckoo populations, study of cuckoos versus meadow pipit broods in heather or *Molinia*-dominated uplands would shed light on the role of habitat type in discrepancy in resource requirements between cuckoo and host. Following combined observation of nest visits and habitat use in the present study, the use of cameras in close proximity to nests is recommended to augment the methodology reported, to allow greater human vantage point distance for observing foraging habitat use by the host pair without loss of data on provisioning visit rate and prey load size. The DNA-based approach to diet analysis in the present study allowed fine-scale ability to detect and identify taxa in nestling diet. However this method indirectly detects

prey through DNA in remains compared to direct visually detection in previous diet studies using faeces, and counts of prey items could not be made. In future studies it is here recommended a randomised selection of samples is directed instead into visual microscopy to provide a supporting dataset of directly observed prey. Studies comparing provisioning rate have generally made no reference to the cuckoo population trend for their study area. The present study area is situated in the south-west region of England where cuckoos have severely declined since 1994 (Harris *et al.* 2018), almost every 20 km square in the immediate vicinity reports declines in cuckoos between 1988-91 and the 2007-11 bird atlases (Balmer *et al.* 2013), and targeted periodic moorland bird surveys in the area suggest cuckoos have declined since the 1970s (Booker *et al.* 2018). Cuckoo abundance at the study area itself was recorded by standardised surveys over three years coinciding with fieldwork for this study but this is unsuitable for trend estimation. It is recommended that future cuckoo ecology studies access all possible trend information for cuckoos local to study areas to provide population context to study findings.

Key cuckoo prey taxa are widely reported to have collapsed under intensification of land management

The present study's analysis of breeding season diet of the common cuckoo on Dartmoor included a high frequency of moth larvae (caterpillars), especially lasiocampid moths, previously reported as frequent cuckoo prey (reviewed Wyllie 1981, Cramp 1988). Juvenile cuckoo diet, from photographic images, included large, often chemically or physically defensive caterpillar species, of similar morphology to those consumed by adults. However adult faecal samples also had high frequency of occurrence of Orthoptera, and chloropid, rhagionid and tipulid flies (Diptera), not previously described as important in the cuckoo diet. While diet of the cuckoo is indicated to be less specialist than previously suggested, the concern remains that key prey are primarily associated with semi-natural habitats and are documented as having declined in the wake of large-scale intensification of land management.

Declines in a significant proportion of larger sized moth species (‘macro-moths’) are well documented in Britain (reviewed Fox 2013). Distribution changes in a significant proportion of British moths are consistent with hypothesised vulnerability to land use intensification. Species with the largest geographic ranges showed significant declines in southern Britain where there has been significant increase in both urban and arable land cover in the same period (Fox *et al.* 2014). Moths whose larval foodplants indicated close associations with open habitats low in nitrogen, declined more negatively in Britain than other moths, suggesting that agricultural intensification, the main source of nitrogen deposition into open habitats, is a driver of decline in moth populations (Fox *et al.* 2014). Increased field sizes remove traditional boundaries such as hedgerows, which have been reported to hold more than double the abundance of moths found in the centre of the same fields (Merckx *et al.* 2009). Conversion of grasslands to arable land, a shift to monoculture crops and removal of uncropped margin area largely or completely eliminates the food plants of Lepidoptera in fields. Oak eggar, fox moth *Macrothylacia rubi*, brown-tail moth *Euproctis chrysorrhoea*, and *Aglais* and *Vanessa* butterflies found in the cuckoo diet in the present study have foodplants commonly found in field margins and hedgerows often lost during field enlargement (or unsympathetically managed with cutting and flailing machinery). Annual or more frequent cutting of hedgerows and field margins prevents many moth species from completing a ful life cycle (Merckx *et al.* 2009). Spraying of agrochemicals such as pesticides in arable land is likely to impact ground feeding larvae (and other insects) in spring when machinery operates at low crop height, while in summer the raised height of spraying is more likely to impact hedgerow-feeding larvae that could include oak eggar, fox moth and brown-tail moth in this period (Dover 2019).

Declines in moths and butterflies have been greatest in arable areas, (Dover 2019) but intensification of grasslands for pasture is also concluded to impact on moths. Drinker moths *E. potatoria* feed at the larval stage on a range of grasses and reeds (Waring & Townsend 2017) that are likely to be outcompeted or grazed out of improved grasslands by reseeding or high density stocking of grazers, respectively (Fuller 1987). Cinnabar *Tyria jacobaeae* larvae were frequently recorded prey of juvenile cuckoos in the photographic analysis. This

species is relatively common in lowlands, especially grasslands with bare earth patches including intensively grazed pastoral agricultural land, as its larval foodplant is common ragwort *Senecio jacobaea*. However this plant is controlled and named under the Weeds Act 1959 and the Ragwort Control Act 2003 due to its toxicity to livestock, especially horses. This legislation does not entail eradication but places responsibility on landowners to control of the spread of the plant "where this presents a high risk of poisoning horses and livestock or spreading to fields for the production of forage" (DEFRA 2007). The subjective nature of this has in practice led to demands that ragwort be removed even from roadside verges (Barnes 2011). While ragwort is only palatable and therefore likely to cause harm when dried out (e.g. in forage), it is often removed while in flower by pulling or cutting (New Forest National Park Authority 2018, Suffolk Wildlife Trust 2018). Not only does this remove available foodplant for cinnabar moths from lowland habitats, but these methods are also less effectual control measures than allowing the plant to set seed and die, and maintaining a level unbroken sward where seeds cannot establish (Crawley 2005). The Centre for Ecology and Hydrology's Countryside Survey reported a decline in ragwort in the UK between 1998 and 2007 (Carey *et al.* 2008), although whether this is mainly driven by control legislation is not clear.

Field margins and hedgerows in pastoral and arable farmland may constitute the only semi-natural habitat in the landscape, but many are poor quality and fragmented (Dover 2019). In addition to providing food plants for some species, hedgerows provide a wider diversity of moths and butterflies with egg laying, roosting, pupation and wintering habitat, plus corridors for movement and shelter from wind (Dover *et al.* 2019). Field margins are areas of non-crop habitat within fields, and wider margins have been shown to support greater abundance of moths, and act as a source habitat increasing abundance within the cropped area (Merckx *et al.* 2009). Trees in hedgerows are additions that benefit some species, including drinker moth (Merckx *et al.* 2010, Merckx & Macdonald 2015). Hedgerows with trees and wide semi-natural field margins were associated with higher abundance of moths, most notably when these were widespread and connected within the landscape, suggesting prominence of continuous semi-natural habitat benefits moth abundance (Merckx &

Macdonald 2015). If hedgerows are increasingly fragmented, they predominantly benefit less mobile, shrub and tree-feeding moth species (Merckx & Macdonald 2015). Farm woodlands have also been shown to be significant refugia for moths, and in a focal study of this habitat moth species richness primarily related to woodland species richness (Usher & Keiller 1998).

Percentage change in adult abundance of moths taken as larvae by cuckoos has been significantly more positive in semi-natural grasslands than in arable land or improved grasslands (Denerley *et al.* 2018). In analysis carried out in Devon for the present study, several moth species documented as cuckoo prey had higher capture probability in largely semi-natural core upland areas than in lowland areas where intensive land use is more prominent. Some species found in this study are more confined to heath and semi-natural grasslands by their larval food plants, such as heath rustic *Xestia agathina*, brown silver-line *P. chlorosata*, *Pseudoterpna sp.* and emperor moth *Saturnia pavonia*. Experiments with grazing pressure in upland *Molinia*-dominated grasslands indicated low intensity sheep grazing (<1 animal ha^{-1}) of grassland plots was associated with highest abundance and species richness of moths (Littlewood 2008). This corresponded to preferred grazing pressure of foraging meadow pipits at the same site (Vendenberghe *et al.* 2009). Drinker, fox moth and oak (northern) eggar *L. quercus* were captured in small numbers but all occurred in greatest abundance in the ungrazed control plots (Littlewood 2008).

Many species of Lepidoptera and Orthoptera lay eggs into soil (Benton 2012) therefore cultivation such as ploughing may have survival impacts on eggs and early instars. A reduction in geographic range has been reported for half of studied Orthoptera species during the 20^{th} century (Robinson and Sutherland 2002) although this has not been directly linked to changes in land management. However, intensive fertilizer application, mowing and grazing are associated with poor orthopteran diversity and abundance (Chisté *et al.* 2016), and conversely orthopteran populations are relatively abundant and diverse in more semi-natural and less intensively managed areas of agricultural landscapes (Marini *et al.* 2008, Rodríguez & Bustamante 2008, Weking *et al.* 2016). Orthopterans with a north-western range limit within Britain have reportedly declined despite having previously been expected to increase under

warming spring temperatures (Sutton *et al.* 2017). This suggests such species are declining due to habitat factors.

Study of abundance and diversity of Diptera relative to land management is limited to narrow species assemblages (Paquette *et al.* 2013) or specific families e.g. Syrphidae (Schweiger *et al.* 2007). Tipulidae at the larval stage are an agricultural pest in the UK, feeding on roots of grasses including in improved grasslands, and there is evidence that larval populations are positively associated with fertiliser application in the previous year (Vickery *et al.* 2001). Rhagionidae are associated with more mixed herb, shrub and woodland habitat. Chloropidae feed on rushes and sedges as larvae and therefore are expected to benefit from unimproved grasslands with relatively diverse herb vegetation. Long term population monitoring of Dipteran populations is lacking but generalist insect monitoring suggests declines in flying insect abundance. Hallmann *et al.* (2017) recorded a 75% decline in flying insect biomass, in nature-allocated areas in a human-dominated lowland landscape. The authors acknowledged few other standardised time series of insect biomass exist for comparison, but the landscape in which data was collected is a similar composition of agricultural and urban land with dispersed natural areas as is found in many areas of Western Europe. In Britain, the Rothamsted Insect Survey used suction traps to monitor aerial insect abundance, and detected a mild decline at one site only (Shortall *et al.* 2009), but the taxonomic range likely to be captured in this protocol was much narrower than that achievable with Malaise traps (Hallman *et al.* 2017). The study by Hallman *et al.* (2017) may be an initial indicator of a wider insect population crisis in western Europe. Repeat censuses of insect abundance at sites where this has historically been carried out may assist in quantifying long term population change in additional locations and regions.

Final conclusions and priority actions

At the close of chapter 1, recommendations were made for prioritising research of the common cuckoo, in light of its declining conservation status in many regions of the breeding range and its complex biology. Emphasis was placed in

particular on the importance of a complete-annual-cycle approach to conservation research, as the ultimate means of confidently identifying key drivers of population in the cuckoo and other long-distance migrants. All recommendations given there, and that emphasis, remain. Here however I make additional recommendations for research and conservation management based purely on the key findings of the present study on the breeding grounds in the UK.

Prior to the present study, there has not been conservation emphasis on the implications of habitat and host specificity of female, and possibly male, cuckoos. **There is reason to suggest that individual cuckoos, and local populations, are specialist parasites which would be incapable of breeding in alternative habitats if their current habitat and host species were lost to land use change.** Assessing the distance to which cuckoos disperse in subsequent seasons, and to what habitat, requires advances and miniaturisation of tracking tags that have yet to take place. The case is the same for directly observing mate choice in cuckoos, and whether it is limited by habitat or ancestral host. It is recommended on current evidence of specialism that cuckoos parasitising nests in different habitats are considered from a conservation perspective as distinct populations. In the context of cuckoos in uplands, predominantly covered in this study, grazing is required in upland semi-natural grassland and heath to create areas of short grass (<30 cm) as key host foraging habitat. Grazer density of less than 1 sheep per hectare is indicated by the literature to benefit both meadow pipit foraging and moth abundance (Littlewood *et al.* 2008, Vandenberghe *et al.* 2009). However securing grazing in uplands at a low density of animals at the same time as preserving habitat heterogeneity requires reconciliation of viewpoints and financial interests of various upland stakeholders, and for low intensity grazing to remain viable in the uplands. Trees presence was a significant factor in meadow pipit foraging as well as in adult cuckoo breeding and feeding behaviours. On this basis the study recommends use of exclosure of grazers or enclosures of trees at some grazed sites to conserve tree regeneration and growth. Specific surveys using paired sites are recommended to gauge the importance of tree presence and density to cuckoo occurrence. Following

preliminary observations of the importance and types of existing perches used by cuckoos, research is recommended that tests the effect of installing artificial perches on cuckoo breeding presence in upland areas lacking trees.

Cuckoo prey taxa detected in the present study have ecological requirements at odds with intensive land management, similarly to the two most frequent host species the meadow pipit and reed warbler. **The study suggests that decline in both host and prey resource was the mechanism by which cuckoos became increasingly dissociated from agricultural land during the twentieth century**. Many regions of Britiain in which cuckoos are thriving have large scale but largely aesthetic-oriented National Park or Area of Outstanding Natural Beauty designations (Balmer *et al.* 2013). These areas are landscape-scale examples of land-sparing with agriculture (mostly improved grassland). Cuckoos are additionally increasing in Britain in the expanses of semi-natural habitat within Scotland (Massimino *et al.* 2017, Harris *et al.* 2018), as also seen in Finland (Laaksonen and Lehikoinen 2013). Understanding what aspect of these low-management areas benefits cuckoos, would aid in determining whether management of landscapes with greater human presence could be effective in conserving or restoring cuckoo populations. Research of moths in agricultural landscapes show that their abundance has been successfully increased by widening of field margins, increasing the floristic quality of seed mixes, and rotational cutting of field margins (once every 2-3 years) (Merckx *et al.* 2009). Moth abundance and species richness have increased under agri-environment schemes and following a shift from conventional to organic farming at study sites (Fox *et al.* 2013), but further research is required to identify the specific factors underlying these apparent effects. Complete conversion of either conventional arable fields or improved pasture to semi-natural grassland increased moth species richness and abundance, but hedgerows had no significant effect in this study (Fuentes-Montemayor *et al.* 2011). Hedgerows appear to be variable in their beneficial impact on moths, relating to context within the landscape and between moth species with different daily mobility (Merckx & Macdonald 2015). Orthoptera are indicated to benefit from fallow areas in agricultural land as well as grassland (Rodríguez & Bustamante 2008) perhaps as part of a wider preference for a mosaic of habitats including many

resulting from less intensive human management such as infrequent mowing (Sergeev 1998). Conservation and restoration of key cuckoo prey moth and Orthoptera populations is overall suggested to relate to increasing the area of non-crop and semi-natural habitat in lowland agricultural areas.

Cuckoos rely on available habitat in Britain from their arrival in April not only for hosts and food sources during breeding but possibly for food sources for initially entering breeding condition. The recommendation is here repeated that renewed data on body condition of cuckoos at varying stages of the breeding season, and indeed annual cycle, would give invaluable insight to how respective areas of habitat and migratory stages influence cuckoo survival.

The present study focused on cuckoos in the relative stronghold of upland grasslands, and pursued questions on nestling ecology in addition to adult ecology. Additional studies of nestling cuckoo diet relative to host broods is recommended, and for further investigating the effect of land use the ideal next step would be to study cuckoos in meadow pipit nests in contrasting habitat such as uplands in Scotland, or lowland heaths in western Europe. Having successfully applied DNA sequencing to the study of adult cuckoo diet in uplands, a key next step is applying this to cuckoos in remaining lowland populations, to establish what prey and foraging habitats are supporting these populations. Further research of the use of other habitats by cuckoos in upland areas such as woodland, is required. This may be possible using PTT tags deployed in the ongoing BTO tracking study, but relatively few upland breeding birds are tagged (Hewson *et al.* 2016). There is also a need to better understand habitat use by cuckoos remaining in the lowlands. Confirmation of the host, diet and habitat use of cuckoos remaining in farmland (Denerley 2014) could provide indication of resources that may be readily reintroduced to wider areas of the lowlands.

Findings within the present study on the adult cuckoo diet, the ecology of key prey species, and the foraging preferences and nestling provisioning behaviour of the host species suggest that conservation of invertebrate biodiversity in grasslands, through the maintenance of structural heterogeneity from short grass patches through diverse herb and shrub vegetation to scattered trees at

the field scale, also results in beneficial habitat structure for host breeding populations, cuckoo parasitism of host nests and cuckoo foraging; the three essential requirements of cuckoo breeding populations.

References

Anderson, R. (2003) *Open ground in upland forests: a review of its potential as wildlife habitat and appropriate management methods*. Available at: https://www.forestry.gov.uk/website/pdf.nsf/b591cb1aa3d9d9ac802570ec004f557d/d7a63ce6e4d131c7802574c80038fa86/$FILE/open_ground_review_2003.pdf.

Barker, A. M., Brown, N. J., and Reynolds, C. J. M. (1999) Do host-plant requirements and mortality from soil cultivation determine the distribution of graminivorous sawflies on farmland? *Journal of Applied Ecology* 36: 271–282.

Barnes, A. (2011) *Authority Meeting 29th September 2011 Chief Executive's Report*, New Forest National Park Authority 386/11.

Benton, T. (2012). *Grasshoppers and Crickets (Collins New Naturalist Library, Book 120)*. HarperCollins UK.

Braun-Blanquet, J. (1932) Plant sociology. The study of plant communities. New York: McGraw-Hill.

Brooke, M. D. L., and Davies, N. B. (1989) Provisioning of nestling cuckoos *Cuculus canorus* by reed warbler *Acrocephalus scirpaceus* hosts. *Ibis* 131(2): 250–256.

Carey, P. D., Wallis, S., Chamberlain, P. M., Cooper, A., Emmett, B. A., Maskell, L. C., McCann, T., Murphy, J., Norton, L. R., Reynolds, B., Scott, W. A., Simpson, I. C., Smart, S. M., and Ullyett, J. M. (2008) *UK Results from 2007 Countryside Survey*. (June 2009.). Centre for Ecology & Hydrology.

Chisté, M. N., Mody, K., Gossner, M. M., Simons, N. K., Köhler, G., Weisser, W. W., and Blüthgen, N. (2016) Losers, winners and opportunists: How grassland land-use intensity affects orthopteran communities. *Ecosphere* 7(11): e01545.

Cramp, S. (1988) *Handbook of the birds of Europe, the Middle East and North Africa: the birds of the western Palearctic.* Oxford, UK: Oxford University Press.

Crawley, M. J. (2005) *The flora of Berkshire: including those parts of modern Oxfordshire that lie to the south of the river Thames; with accounts of charophytes, ferns, flowering plants, bryophytes, lichens and non-lichenized fungi.* Brambleby books.

DEFRA (2007) *Code of Practice on How to Prevent the Spread of Ragwort - PB9840 revised March 2007*. Department for Environment, Food and Rural Affairs, London.

Douglas, D. J. T., Evans, D. M., and Redpath, S. M. (2008) Selection of foraging habitat and nestling diet by Meadow Pipits *Anthus pratensis* breeding on intensively grazed moorland. *Bird Study* 55(3): 290–296.

Dover, J.W. ed., (2019). *The Ecology of Hedgerows and Field Margins*. Routledge.

Evans, D. M., Redpath, S. M., Evans, S. A., Elston, D. A., Gardner, C. J., Dennis, P. and Pakeman, R. J. (2006) 'Low intensity, mixed livestock grazing improves the breeding abundance of a common insectivorous passerine', *Biology Letters* 2(4): 636–638.

Fox, R. (2013) The decline of moths in Great Britain: A review of possible causes. *Insect Conservation and Diversity* 6(1): 5–19.

Fox, R., Parsons, M.S., Chapman, J.W., Woiwod, I.P., Warren, M.S. and Brooks, D.R. (2013) *The State of Britain's Larger Moths 2013.* Butterfly Conservation and Rothamsted Research, Wareham, Dorset, UK

Fox, R., Oliver, T. H., Harrower, C., Parsons, M. S., Thomas, C. D., and Roy, D. B. (2014) Long-term changes to the frequency of occurrence of British moths are consistent with opposing and synergistic effects of climate and land-use changes. *Journal of Applied Ecology* 51(4): 949–957.

Fuentes-Montemayor, E., Goulson, D., and Park, K. J. (2011) The effectiveness of agri-environment schemes for the conservation of farmland moths: Assessing

the importance of a landscape-scale management approach. *Journal of Applied Ecology* 48(3): 532–542.

Fuller, R. M. (1987) The changing extent and conservation interest of lowland grasslands in England and Wales: A review of grassland surveys 1930–1984. *Biological Conservation* 40(4): 281–300.

Harrison, M. (2016) Rewilding National Parks: Moor than meets the eye, *ECOS*, 37 (1): 43-47

Laaksonen, T., and Lehikoinen, A. (2013) Population trends of boreal birds: continuing declines in long-distance migrants, agricultural and northern species. *Biological Conservation* 168: 99–107.

Lack, D. (1933). Habitat selection in birds. With special reference to the effects of afforestation on the Breckland avifauna. *The Journal of Animal Ecology* 239-262.

Littlewood, N. A. (2008) Grazing impacts on moth diversity and abundance on a Scottish upland estate. *Insect Conserv Divers* 1: 151–160.

Marini, L., Fontana, P., Scotton, M., and Klimek, S. (2008) Vascular plant and Orthoptera diversity in relation to grassland management and landscape composition in the European Alps. *Journal of Applied Ecology* 45(1): 361–370.

Massimino, D., Woodward, I.D., Hammond, M.J., Harris, S.J., Leech, D.I., Noble, D.G., Walker, R.H., Barimore, C., Dadam, D., Eglington, S.M., Marchant, J.H., Sullivan, M.J.P., Baillie, S.R. and Robinson, R. A. (2017) *BirdTrends 2017: trends in numbers, breeding success and survival for UK breeding birds. Research Report 704.* Thetford. Available at: www.bto.org/birdtrends.

Merckx, T., and Macdonald, D. W. (2015) Landscape-scale conservation of farmland moths. In Macdonald, D. W. and Feber, R. E. (Eds.), *Wildlife Conservation on Farmland. Volume 1: Managing for Nature on Lowland Farms*. Oxford: Oxford University Press doi:10.1093/acprof.

Merckx, T., Feber, R. E., Dulieu, R. L., Townsend, M. C., Parsons, M. S., Bourn, N. A. D., Riordan, P., and Macdonald, D. W. (2009) Effect of field margins on

moths depends on species mobility: Field-based evidence for landscape-scale conservation. *Agriculture, Ecosystems and Environment* 129(1–3): 302–309.

Merckx, T., Feber, R.E., Mclaughlan, C., Bourn, N.A., Parsons, M.S., Townsend, M.C., Riordan, P. and Macdonald, D.W., (2010). Shelter benefits less mobile moth species: the field-scale effect of hedgerow trees. *Agriculture, ecosystems & environment* 138(3-4): 147-151.

Monbiot, G. (2016) Meet the conservationists who believe that burning is good for wildlife, *The Guardian* 14 Jan 2016 Available at: https://www.theguardian.com/environment/georgemonbiot/2016/jan/14/swaling-is-causing-an-environmental-disaster-on-britains-moors

Moss, D., Taylor, P.N. and Easterbee, N., (1979). The effects on song-bird populations of upland afforestation with spruce. *Forestry: An International Journal of Forest Research* 52(2): 129-150.

Nakamura, H. and Miyazawa, Y. (1997) 'Movements, space use and social organisation of radio tracked common cuckoos during the breeding season in Japan', *Japanese Journal of Ornithology*, 46: 23–54.

New Forest National Park Authority (2018) *Pasture management guidelines for equine owners*.

North York Moors National Park Authority (2012) *North York Moors National Park Management Plan: Summary of consultation responses*. York.

Paquette, S.R., Garant, D., Pelletier, F. and Bélisle, M., (2013). Seasonal patterns in tree swallow prey (Diptera) abundance are affected by agricultural intensification. *Ecological Applications 23*(1):122-133.

Pearce-Higgins, J.W., Yalden, D.W. and Whittingham, M.J., (2005). Warmer springs advance the breeding phenology of golden plovers *Pluvialis apricaria* and their prey (Tipulidae). *Oecologia* 143(3): 470-476.

Robinson, R.A. and Sutherland, W.J. (2002) Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*, 39: 157–176.

Rodríguez, C. and Bustamante, J. (2008) ‘Patterns of Orthoptera abundance and lesser kestrel conservation in arable landscapes’, *Biodiversity and Conservation* 17: 1753–1764.

Røskaft, E., Moksnes, A., Stokke, G., Moska, C., and Honza, M. (2002) The spatial habitat structure of host populations explains the pattern of rejection behavior in hosts and parasitic adaptations in cuckoos. *Behavioral Ecology* 13(2): 163–168.

Rowland, C.S.; Morton, R.D.; Carrasco, L.; McShane, G.; O’Neil, A.W.; Wood, C.M. (2017). *Land Cover Map 2015 (25m raster, GB).* NERC Environmental Information Data Centre.

Schweiger, O., Musche, M., Bailey, D., Billeter, R., Diekötter, T., Hendrickx, F., Herzog, F., Liira, J., Maelfait, J.P., Speelmans, M. and Dziock, F., (2007). Functional richness of local hoverfly communities (Diptera, Syrphidae) in response to land use across temperate Europe. *Oikos* 116(3): 461-472.

Scottish Government (2016) *Wildlife Management on Upland and Peatland Sites*, *Scottish Rural Development Programme 2014 - 2020 - Rural Priorities - List of Options*. Available at: https://www2.gov.scot/Topics/farmingrural/SRDP/RuralPriorities/Options/UplandandPeatlandSites (Accessed: 13 February 2019).

Sergeev, M. G. (1998) Conservation of orthopteran biological diversity relative to landscape change in temperate Eurasia. *Journal of Insect Conservation* 2: 247–252.

Shortall, C. R., Moore, A., Smith, E., Hall, M. J., Woiwod, I. P., and Harrington, R. (2009) Long-term changes in the abundance of flying insects. *Insect Conservation and Diversity* 2(4): 251–260.

Smith, A. A., Redpath, S. M., Campbell, S. T., and Thirgood, S. J. (2001) Meadow pipits , red grouse and the habitat characteristics of managed grouse moors. *Journal of Applied Ecology* 38: 390–400.

Suffolk Wildlife Trust (2019) *Ragwort Management*. Available at: https://www.suffolkwildlifetrust.org/ragwort-management.

Sutton, P. G., Beckmann, B. C. and Nelson, B. (2017) 'The Current Status of Orthopteroid Insects in Britain and Ireland', *Atropos*, 59, pp. 6–35. Available at: www.atropos.info.

Usher, M.B. and Keiller, S.W., 1998. The macrolepidoptera of farm woodlands: determinants of diversity and community structure. *Biodiversity & Conservation* 7(6): 725-748.

Vandenberghe, C., Prior, G., Littlewood, N. a., Brooker, R., and Pakeman, R. (2009) Influence of livestock grazing on meadow pipit foraging behaviour in upland grassland. *Basic and Applied Ecology* 10(7): 662–670.

Vickery, J. A., Tallowin, J. R., Feber, R. E., Asteraki, E. J., Atkinson, P. W., Fuller, R. J., and Brown, V. K. (2001) The management of lowland neutral grasslands in Britain: effects of agricultural practices on birds and their food resources. *Journal of Applied Ecology* 38(3): 647–664.

Vogl, W., Taborsky, B., Teuschl, Y., Taborsky, M., and Honza, M. (2004) Habitat and space use of European cuckoo females during the egg laying period. *Behaviour* 141(7): 881–898.

Weking, S., Kämpf, I., Mathar, W., and Hölzel, N. (2016) Effects of land use and landscape patterns on Orthoptera communities in the Western Siberian forest steppe. *Biodiversity and Conservation* 25(12): 2341–2359.

Appendices

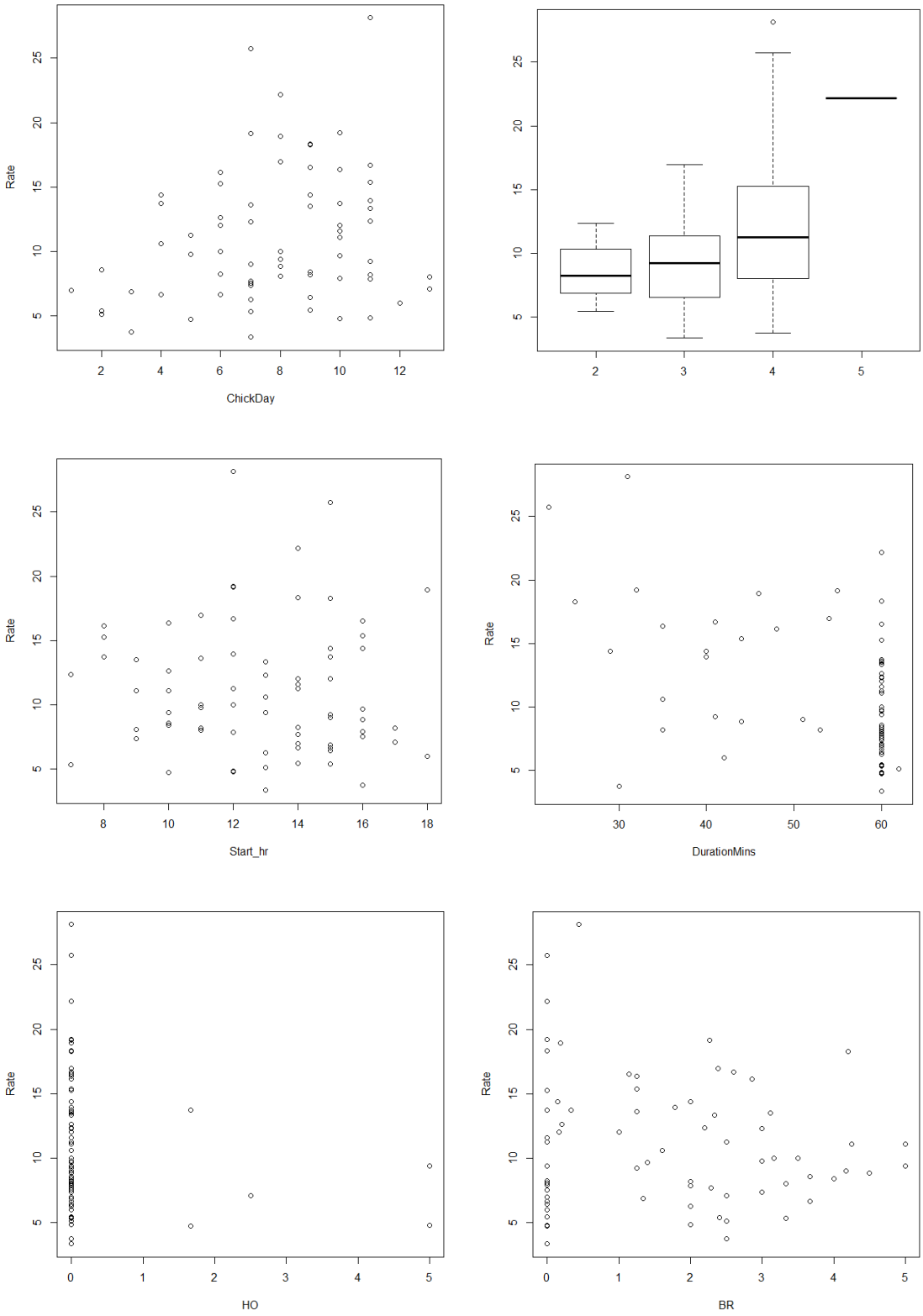
Appendix 1. Alignment of database common cuckoo and meadow pipit COI mitochondrial DNA sequences, showing complements of vertebrate and avian COI primers and their location

```
gi|817249888|emb|LN734592.1|      ------------------------------TCTATACTTAATCTTTGGTGCTTGAGCCGG
gi|817249799|emb|LN734581.1|      ------------------------------TCTATACTTAATCTTTGGTGCTTGAGCCGG
gi|817249797|emb|LN734580.1|      ------------------------------TCTATACTTAATCTTTGGTGCTTGAGCCGG
gi|817249795|emb|LN734579.1|      ------------------------------TCTATACTTAATCTTTGGTGCTTGAGCCGG
gi|817249792|emb|LN734578.1|      ------------------------------TCTATACTTAATCTTTGGTGCTTGAGCCGG
gi|1039305146|gb|KX283112.1|      -------------------------------------TAATCTTTGGTGCTTGAGCCGG
gi|292388828|gb|GU571254.1|       ------------------------------CTATACCTAATCTTCGGCGCATGAGCAGG
gi|292388834|gb|GU571257.1|       ---------PASSERF1----------------------ATCTTCGGCGCATGAGCAGG
gi|292388830|gb|GU571255.1|       ------AACCAACCACAAAGATATCGGGACCCTATACCTAATCTTCGGCGCATGAGCAGG
gi|292389784|gb|GU571732.1|       ----------------------------------CTAATCTTCGGCGCATGAGCAGG
gi|257153735|gb|GQ481350.1|       ------------------------------------------------------------GG
gi|257153741|gb|GQ481353.1|       ------------------------------CCTATACCTAATCTTCGGCGCATGAGCAGG
gi|257153743|gb|GQ481354.1|       ----------BIRDF1-------------CCTATACCTAATCTTCGGCGCATGAGCAGG
gi|292388832|gb|GU571256.1|       TTTTTCTCCAACCCACAAAGACATTGGCACCCTATACCTAATCTTCGGCGCATGAGCAGG
gi|361050404|gb|JN801265.1|       ------------------------------------------------------------G
gi|292389782|gb|GU571731.1|       ----------------------------------CTAATCTTCGGCGCATGAGCAGG
gi|257153739|gb|GQ481352.1|       ------------------------------CCTATACCTAATCTTCGGCGCATGAGCAGG
gi|257153737|gb|GQ481351.1|       ------------------------------CCTATACCTAATCTTCGGCGCATGAGCAGG
                                                                                            *

gi|817249888|emb|LN734592.1|      TATGGTAGGAACAGCCCTGAGCCTACTTATTCGTGCAGAACTAGGACAACCAGGAACCCT
gi|817249799|emb|LN734581.1|      TATGGTAGGAACAGCCCTGAGCCTACTTATTCGTGCAGAACTAGGACAACCAGGAACCCT
gi|817249797|emb|LN734580.1|      TATGGTAGGAACAGCCCTGAGCCTACTTATTCGTGCAGAACTAGGACAACCAGGAACCCT
gi|817249795|emb|LN734579.1|      TATGGTAGGAACAGCCCTGAGCCTACTTATTCGTGCAGAACTAGGACAACCAGGAACCCT
gi|817249792|emb|LN734578.1|      TATGGTAGGAACAGCCCTGAGCCTACTTATTCGTGCAGAACTAGGACAACCAGGAACCCT
gi|1039305146|gb|KX283112.1|      TATGGTAGGAACAGCCCTGAGCCTACTTATTCGTGCAGAACTAGGACAACCAGGAACCCT
gi|292388828|gb|GU571254.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATTCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|292388834|gb|GU571257.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|292388830|gb|GU571255.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|292389784|gb|GU571732.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|257153735|gb|GQ481350.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|257153741|gb|GQ481353.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|257153743|gb|GQ481354.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|292388832|gb|GU571256.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAAATAGGCCAACCCGGAGCTCT
gi|361050404|gb|JN801265.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|292389782|gb|GU571731.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|257153739|gb|GQ481352.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
gi|257153737|gb|GQ481351.1|       AATAGTAGGCACTGCCCTGAGCCTCCTCATCCGAGCAGAACTAGGCCAACCCGGAGCTCT
                                   ** ***** ** *********** ** ** ** ****** **** ***** *** * **

gi|817249888|emb|LN734592.1|      CCTCGGAGACGACCAAATCTACAATGTAATCGTTACAGCACATGCTTTCGTAATAATTTT
gi|817249799|emb|LN734581.1|      CCTCGGAGACGACCAAATCTACAATGTAATCGTTACAGCACATGCTTTCGTAATAATTTT
gi|817249797|emb|LN734580.1|      CCTCGGAGACGACCAAATCTACAATGTAATCGTTACAGCACATGCTTTCGTAATAATTTT
gi|817249795|emb|LN734579.1|      CCTCGGAGACGACCAAATCTACAATGTAATCGTTACAGCACATGCTTTCGTAATAATTTT
gi|817249792|emb|LN734578.1|      CCTCGGAGACGACCAAATCTACAATGTAATCGTTACAGCACATGCTTTCGTAATAATTTT
gi|1039305146|gb|KX283112.1|      CCTCGGAGACGACCAAATCTACAATGTAATCGTTACAGCACATGCTTTCGTAATAATTTT
gi|292388828|gb|GU571254.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
gi|292388834|gb|GU571257.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
gi|292388830|gb|GU571255.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
gi|292389784|gb|GU571732.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
gi|257153735|gb|GQ481350.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCNTCGTAATGATCTT
gi|257153741|gb|GQ481353.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACNGCCCACGCCTTCGTAATGATCTT
gi|257153743|gb|GQ481354.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
gi|292388832|gb|GU571256.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
gi|361050404|gb|JN801265.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
gi|292389782|gb|GU571731.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
gi|257153739|gb|GQ481352.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
gi|257153737|gb|GQ481351.1|       CCTAGGAGACGACCAAGTCTACAACGTAGTCGTCACTGCCCACGCCTTCGTAATGATCTT
                                  *** ************ ******* *** **** ** ** ** **  ******* ** **

gi|817249888|emb|LN734592.1|      CTTTATAGTTATGCCAATCATAATTGGAGGATTCGGAAACTGACTAGTCCCACTTATAAT
gi|817249799|emb|LN734581.1|      CTTTATAGTTATGCCAATCATAATTGGAGGATTCGGAAACTGACTAGTCCCACTTATAAT
gi|817249797|emb|LN734580.1|      CTTTATAGTTATGCCAATCATAATTGGAGGATTCGGAAACTGACTAGTCCCACTTATAAT
gi|817249795|emb|LN734579.1|      CTTTATAGTTATGCCAATCATAATTGGAGGATTCGGAAACTGACTAGTCCCACTTATAAT
gi|817249792|emb|LN734578.1|      CTTTATAGTTATGCCAATCATAATTGGAGGATTCGGAAACTGACTAGTCCCACTTATAAT
gi|1039305146|gb|KX283112.1|      CTTTATAGTTATGCCAATCATAATTGGAGGATTCGGAAACTGACTAGTCCCACTTATAAT
gi|292388828|gb|GU571254.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|292388834|gb|GU571257.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|292388830|gb|GU571255.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|292389784|gb|GU571732.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|257153735|gb|GQ481350.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
```

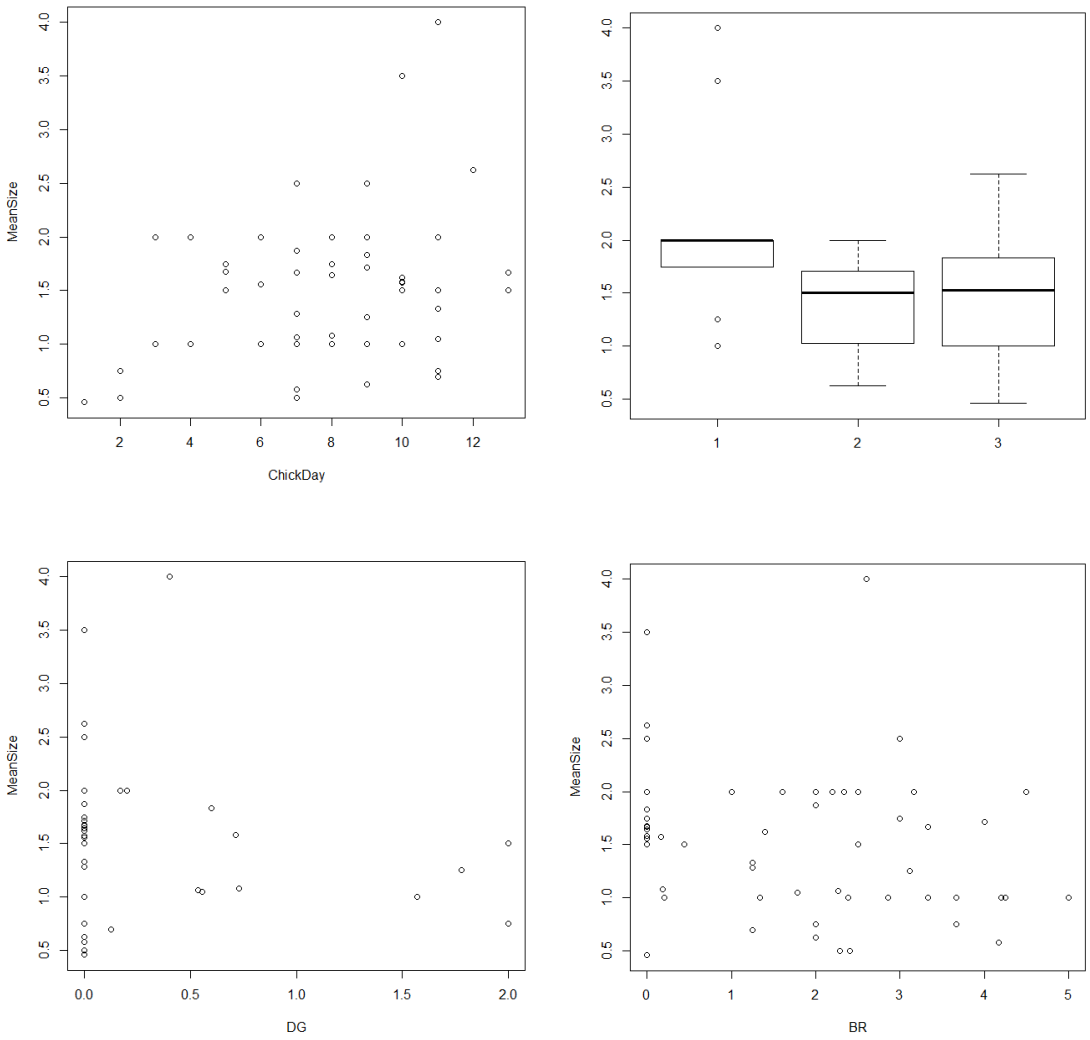
```
gi|257153741|gb|GQ481353.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|257153743|gb|GQ481354.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|292388832|gb|GU571256.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|361050404|gb|JN801265.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|292389782|gb|GU571731.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|257153739|gb|GQ481352.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
gi|257153737|gb|GQ481351.1|       CTTCATGGTCATACCCATCATGATCGGAGGATTCGGAAACTGACTAGTCCCCCTAATAAT
                                  *** ** ** ** ** ***** ** ************************** ** *****

                                      AvMiF1
gi|817249888|emb|LN734592.1|      TGGTGCCCCAGACATAGCATTCCCACGCATAAACAACATGAGCTTCTGACTTCTCCCCCC
gi|817249799|emb|LN734581.1|      TGGTGCCCCAGACATAGCATTCCCACGCATAAACAACATGAGCTTCTGACTTCTCCCCCC
gi|817249797|emb|LN734580.1|      TGGTGCCCCAGACATAGCATTCCCACGCATAAACAACATGAGCTTCTGACTTCTCCCCCC
gi|817249795|emb|LN734579.1|      TGGTGCCCCAGACATAGCATTCCCACGCATAAACAACATGAGCTTCTGACTTCTCCCCCC
gi|817249792|emb|LN734578.1|      TGGTGCCCCAGACATAGCATTCCCACGCATAAACAACATGAGCTTCTGACTTCTCCCCCC
gi|1039305146|gb|KX283112.1|      TGGTGCCCCAGACATAGCATTCCCACGCATAAACAACATGAGCTTCTGACTTCTCCCCCC
gi|292388828|gb|GU571254.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|292388834|gb|GU571257.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|292388830|gb|GU571255.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|292389784|gb|GU571732.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|257153735|gb|GQ481350.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|257153741|gb|GQ481353.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|257153743|gb|GQ481354.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|292388832|gb|GU571256.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|361050404|gb|JN801265.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|292389782|gb|GU571731.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|257153739|gb|GQ481352.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
gi|257153737|gb|GQ481351.1|       CGGAGCCCCGGACATAGCATTCCCCCGAATAAATAACATAAGTTTCTGACTACTTCCACC
                                   ** ***** ************** ** ***** ***** ** ******** ** ** **

gi|817249888|emb|LN734592.1|      ATCCTTCTTACTCTTACTAGCCTCTTCAACAGTAGAAGCGGGAGCAGGAACCGGATGAAC
gi|817249799|emb|LN734581.1|      ATCCTTCTTACTCTTACTAGCCTCTTCAACAGTAGAAGCGGGAGCAGGAACCGGATGAAC
gi|817249797|emb|LN734580.1|      ATCCTTCTTACTCTTACTAGCCTCTTCAACAGTAGAAGCGGGAGCAGGAACCGGATGAAC
gi|817249795|emb|LN734579.1|      ATCCTTCTTACTCTTACTAGCCTCTTCAACAGTAGAAGCGGGAGCAGGAACCGGATGAAC
gi|817249792|emb|LN734578.1|      ATCCTTCTTACTCTTACTAGCCTCTTCAACAGTAGAAGCGGGAGCAGGAACCGGATGAAC
gi|1039305146|gb|KX283112.1|      ATCCTTCTTACTCTTACTAGCCTCTTCAACAGTAGAAGCGGGAGCAGGAACCGGATGAAC
gi|292388828|gb|GU571254.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|292388834|gb|GU571257.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACANTCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|292388830|gb|GU571255.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|292389784|gb|GU571732.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|257153735|gb|GQ481350.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|257153741|gb|GQ481353.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|257153743|gb|GQ481354.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|292388832|gb|GU571256.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|361050404|gb|JN801265.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|292389782|gb|GU571731.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|257153739|gb|GQ481352.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
gi|257153737|gb|GQ481351.1|       ATCATTTCTCCTCCTACTAGCATCCTCCACAATCGAAGCAGGAGTCGGTACAGGCTGAAC
                                  *** **  * *** ******* ** ** *** * ***** ****  ** ** ** *****

                                                                    AvMiR1
gi|817249888|emb|LN734592.1|      AGTATACCCCCCATTAGCCGGCAACTTAGCCCACGCCGCGGGCATCAGTAGACCTAGCCAT
gi|817249799|emb|LN734581.1|      AGTATACCCCCCATTAGCCGGCAACTTAGCCCACGCCGGGGGCATCAGTAGACCTAGCCAT
gi|817249797|emb|LN734580.1|      AGTATACCCCCCATTAGCCGGCAACTTAGCCCACGCCGGGGGCATCAGTAGACCTAGCCAT
gi|817249795|emb|LN734579.1|      AGTATACCCCCCATTAGCCGGCAACTTAGCCCACGCCGGGGGCATCAGTAGACCTAGCCAT
gi|817249792|emb|LN734578.1|      AGTATACCCCCCATTAGCCGGCAACTTAGCCCACGCCGGGGGCATCAGTAGACCTAGCCAT
gi|1039305146|gb|KX283112.1|      AGTATACCCCCCATTAGCCGGCAACTTAGCCCACGCCGGGGCATCAGTAGACCTAGCCAT
gi|292388828|gb|GU571254.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|292388834|gb|GU571257.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|292388830|gb|GU571255.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|292389784|gb|GU571732.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|257153735|gb|GQ481350.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|257153741|gb|GQ481353.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|257153743|gb|GQ481354.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|292388832|gb|GU571256.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|361050404|gb|JN801265.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|292389782|gb|GU571731.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|257153739|gb|GQ481352.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
gi|257153737|gb|GQ481351.1|       TGTATACCCCCCACTAGCCGGTAACCTAGCCCACGCCGGAGCATCCGTTGATCTAGCAAT
                                   ************ ******* *** ************** ***** ** ** ***** **

gi|817249888|emb|LN734592.1|      CTTCTCCCTACACCTAGCAGGTGTTTCATCAATCCTAGGAGCAATCAACTTCATCACAAC
gi|817249799|emb|LN734581.1|      CTTCTCCCTACACCTAGCAGGTGTTTCATCAATCCTAGGAGCAATCAACTTCATCACAAC
gi|817249797|emb|LN734580.1|      CTTCTCCCTACACCTAGCAGGTGTTTCATCAATCCTAGGAGCAATCAACTTCATCACAAC
gi|817249795|emb|LN734579.1|      CTTCTCCCTACACCTAGCAGGTGTTTCATCAATCCTAGGAGCAATCAACTTCATCACAAC
gi|817249792|emb|LN734578.1|      CTTCTCCCTACACCTAGCAGGTGTTTCATCAATCCTAGGAGCAATCAACTTCATCACAAC
gi|1039305146|gb|KX283112.1|      CTTCTCCCTACACCTAGCAGGTGTTTCATCAATCCTAGGAGCAATCAACTTCATCACAAC
gi|292388828|gb|GU571254.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGGGCAATCAACTTCATCACAAC
gi|292388834|gb|GU571257.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
gi|292388830|gb|GU571255.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
gi|292389784|gb|GU571732.1|       CTTCTCCCTGCACCTAGCCGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
gi|257153735|gb|GQ481350.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
gi|257153741|gb|GQ481353.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
gi|257153743|gb|GQ481354.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
```

```
gi|292388832|gb|GU571256.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
gi|361050404|gb|JN801265.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
gi|292389782|gb|GU571731.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
gi|257153739|gb|GQ481352.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
gi|257153737|gb|GQ481351.1|       CTTCTCCCTGCACCTAGCTGGAATTTCCTCTATCCTAGGCGCAATCAACTTCATCACAAC
                                  ********* ******** **  **** ** ******** ********************

gi|817249888|emb|LN734592.1|      AGCCATCAACATAAAACCTCCCGCACTGTCCCAATACCAAACACCCCTATTCGTATGATC
gi|817249799|emb|LN734581.1|      AGCCATCAACATAAAACCTCCCGCACTGTCCCAATACCAAACACCCCTATTCGTATGATC
gi|817249797|emb|LN734580.1|      AGCCATCAACATAAAACCTCCCGCACTGTCCCAATACCAAACACCCCTATTCGTATGATC
gi|817249795|emb|LN734579.1|      AGCCATCAACATAAAACCTCCCGCACTGTCCCAATACCAAACACCCCTATTCGTATGATC
gi|817249792|emb|LN734578.1|      AGCCATCAACATAAAACCTCCCGCACTGTCCCAATACCAAACACCCCTATTCGTATGATC
gi|1039305146|gb|KX283112.1|      AGCCATCAACATAAAACCTCCCGCACTGTCCCAATACCAAACACCCCTATTCGTATGATC
gi|292388828|gb|GU571254.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|292388834|gb|GU571257.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|292388830|gb|GU571255.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|292389784|gb|GU571732.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|257153735|gb|GQ481350.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|257153741|gb|GQ481353.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|257153743|gb|GQ481354.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTATTTGTCTGATC
gi|292388832|gb|GU571256.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|361050404|gb|JN801265.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|292389782|gb|GU571731.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|257153739|gb|GQ481352.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
gi|257153737|gb|GQ481351.1|       AGCAATCAACATAAAACCTCCCGCCCTCTCACAATATCAAACCCCCCTGTTTGTCTGATC
                                  *** ******************** ** ** ***** ***** ***** ** ** *****

gi|817249888|emb|LN734592.1|      AGTACTTATCACCGCCGTCCTACTCCTATTGTCCCTACCCGTACTAGCCGCCGGTATCAC
gi|817249799|emb|LN734581.1|      AGTACTTATCACCGCCGTCCTACTCCTATTGTCCCTACCCGTACTAGCCGCCGGTATCAC
gi|817249797|emb|LN734580.1|      AGTACTTATCACCGCCGTCCTACTCCTATTGTCCCTACCCGTACTAGCCGCCGGTATCAC
gi|817249795|emb|LN734579.1|      AGTACTTATCACCGCCGTCCTACTCCTATTGTCCCTACCCGTACTAGCCGCCGGTATCAC
gi|817249792|emb|LN734578.1|      AGTACTTATCACCGCCGTCCTACTCCTATTGTCCCTACCCGTACTAGCCGCCGGTATCAC
gi|1039305146|gb|KX283112.1|      AGTACTTATCACCGCCGTCCTACTCCTATTGTCCCTACCCGTACTAGCCGCCGGTATCAC
gi|292388828|gb|GU571254.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|292388834|gb|GU571257.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|292388830|gb|GU571255.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|292389784|gb|GU571732.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|257153735|gb|GQ481350.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|257153741|gb|GQ481353.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|257153743|gb|GQ481354.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|292388832|gb|GU571256.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|361050404|gb|JN801265.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|292389782|gb|GU571731.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|257153739|gb|GQ481352.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
gi|257153737|gb|GQ481351.1|       CGTCCTAATCACCGCAGTACTACTCCTGCTCTCCCTACCGGTACTGGCCGCAGGCATCAC
                                   ** ** ******** ** ********  * ******** ***** ***** ** *****

gi|817249888|emb|LN734592.1|      GATACTACTAACAGATCGCAATCTAAACACCACATTCTTCGACCCAGCAGGAGGAGGTGA
gi|817249799|emb|LN734581.1|      GATACTACTAACAGATCGCAATCTAAACACCACATTCTTCGACCCAGCAGGAGGAGGTGA
gi|817249797|emb|LN734580.1|      GATACTACTAACAGATCGCAATCTAAACACCACATTCTTCGACCCAGCAGGAGGAGGTGA
gi|817249795|emb|LN734579.1|      GATACTACTAACAGATCGCAATCTAAACACCACATTCTTCGACCCAGCAGGAGGAGGTGA
gi|817249792|emb|LN734578.1|      GATACTACTAACAGATCGCAATCTAAACACCACATTCTTCGACCCAGCAGGAGGAGGTGA
gi|1039305146|gb|KX283112.1|      GATACTACTAACAGATCGCAATCTAAACACCACATTCTTCGACCCAGCAGGAGGAGGTGA
gi|292388828|gb|GU571254.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
gi|292388834|gb|GU571257.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTTGACCCAGCAGGAGGAGGAGA
gi|292388830|gb|GU571255.1|       AATACTCCTAACGGACCGTAACCTCAACACCANATTCTTCGACCCNGCAGGAGGAGGAGA
gi|292389784|gb|GU571732.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
gi|257153735|gb|GQ481350.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
gi|257153741|gb|GQ481353.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
gi|257153743|gb|GQ481354.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
gi|292388832|gb|GU571256.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
gi|361050404|gb|JN801265.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
gi|292389782|gb|GU571731.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
gi|257153739|gb|GQ481352.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
gi|257153737|gb|GQ481351.1|       AATACTCCTAACGGACCGTAACCTCAACACCACATTCTTCGACCCAGCAGGAGGAGGAGA
                                   ***** ***** ** ** ** ** ******* ****** ***** *********** **

gi|817249888|emb|LN734592.1|      CCCCGTATTATACCAACACCTATTCTGATTCTTTGGACACCCAGAAGTCTACATCCTAAT
gi|817249799|emb|LN734581.1|      CCCCGTATTATACCAACACCTATTCTGATTCTTTGGACACCCAGAAGTCTACATCCTAAT
gi|817249797|emb|LN734580.1|      CCCCGTATTATACCAACACCTATTCTGATTCTTTGGACACCCAGAAGTCTACATCCTAAT
gi|817249795|emb|LN734579.1|      CCCCGTATTATACCAACACCTATTCTGATTCTTTGGACACCCAGAAGTCTACATCCTAAT
gi|817249792|emb|LN734578.1|      CCCCGTATTATACCAACACCTATTCTGATTCTTTGGACACCCAGAAGTCTACATCCTAAT
gi|1039305146|gb|KX283112.1|      CCCCGTATTATACCAACACCTATTCTG------- COI R--------------------
gi|292388828|gb|GU571254.1|       CCCAGTCCTATACCAACATCTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCCTAAT
gi|292388834|gb|GU571257.1|       CCCAGTCCTATACCAACATCTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCC----
gi|292388830|gb|GU571255.1|       CCCAGTCCTATACCAACATCTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCCTAAT
gi|292389784|gb|GU571732.1|       CCCAGTCCTATACCAACATCTTTTTT----------------------------------
gi|257153735|gb|GQ481350.1|       CCCAGTCCTATACCAACATCTTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCCTAAT
gi|257153741|gb|GQ481353.1|       CCCAGTCCTATACCAACATCTTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCCTAAT
gi|257153743|gb|GQ481354.1|       CCCAGTCCTATACCAACATCTTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCCTAAT
gi|292388832|gb|GU571256.1|       CCCAGTCCTATACCAACATCTTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCCTAAT
gi|361050404|gb|JN801265.1|       CCCAGTCCTATACCAACATCTTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCCTAAT
```

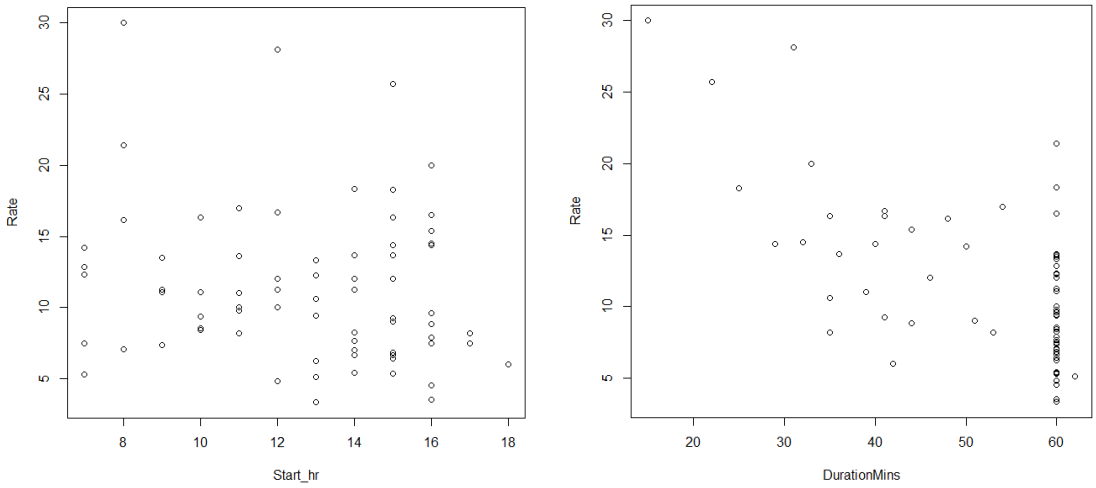
```
gi|292389782|gb|GU571731.1|       CCCAGTCCTATACCAACATCTTTTT----------------------------------
gi|257153739|gb|GQ481352.1|       CCCAGTCCTATACCAACATCTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCCTAAT
gi|257153737|gb|GQ481351.1|       CCCAGTCCTATACCAACATCTTTTTTGATTCTTTGGCCACCCAGAAGTCTACATCCTAAT
                                  *** **  ********** ** **

gi|817249888|emb|LN734592.1|      TCTAC--------------------------
gi|817249799|emb|LN734581.1|      TCTAC--------------------------
gi|817249797|emb|LN734580.1|      TCTAC--------------------------
gi|817249795|emb|LN734579.1|      TCTAC--------------------------
gi|817249792|emb|LN734578.1|      TCTAC--------------------------
gi|1039305146|gb|KX283112.1|     ------------------------------
gi|292388828|gb|GU571254.1|      CCTCCCAGGATTTGGAATTATCTCCCACGTA BIRD R1
gi|292388834|gb|GU571257.1|      ------------------------------
gi|292388830|gb|GU571255.1|      CCTCCCAGGATT------------------
gi|292389784|gb|GU571732.1|      ------------------------------
gi|257153735|gb|GQ481350.1|      CCTC--------------------------
gi|257153741|gb|GQ481353.1|      CCTC--------------------------
gi|257153743|gb|GQ481354.1|      CCTC--------------------------
gi|292388832|gb|GU571256.1|      CCTCCCAGGATTTGGAATTATCTCCCACGTA BIRD R1
gi|361050404|gb|JN801265.1|      CCTC--------------------------
gi|292389782|gb|GU571731.1|      ------------------------------
gi|257153739|gb|GQ481352.1|      CCTC--------------------------
gi|257153737|gb|GQ481351.1|      CCTC--------------------------
```



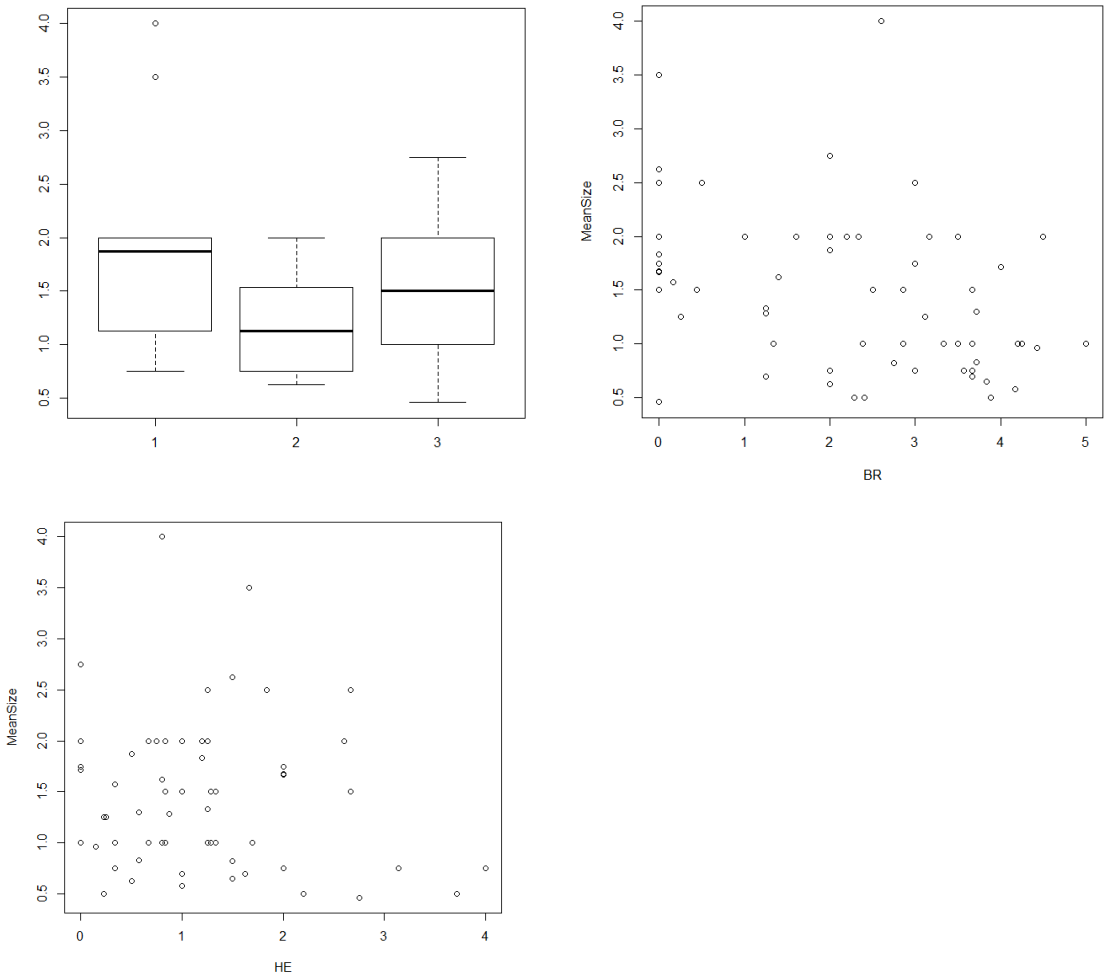
Appendix 2. Plots showing variation in unparasitised nests' provisioning visit rate with nestling age, brood size, start hour, duration, foraging plot (FP) homogeneous tall pastoral grass cover (HO) and FP bracken cover (BR) (Chapter 3 Table 4).



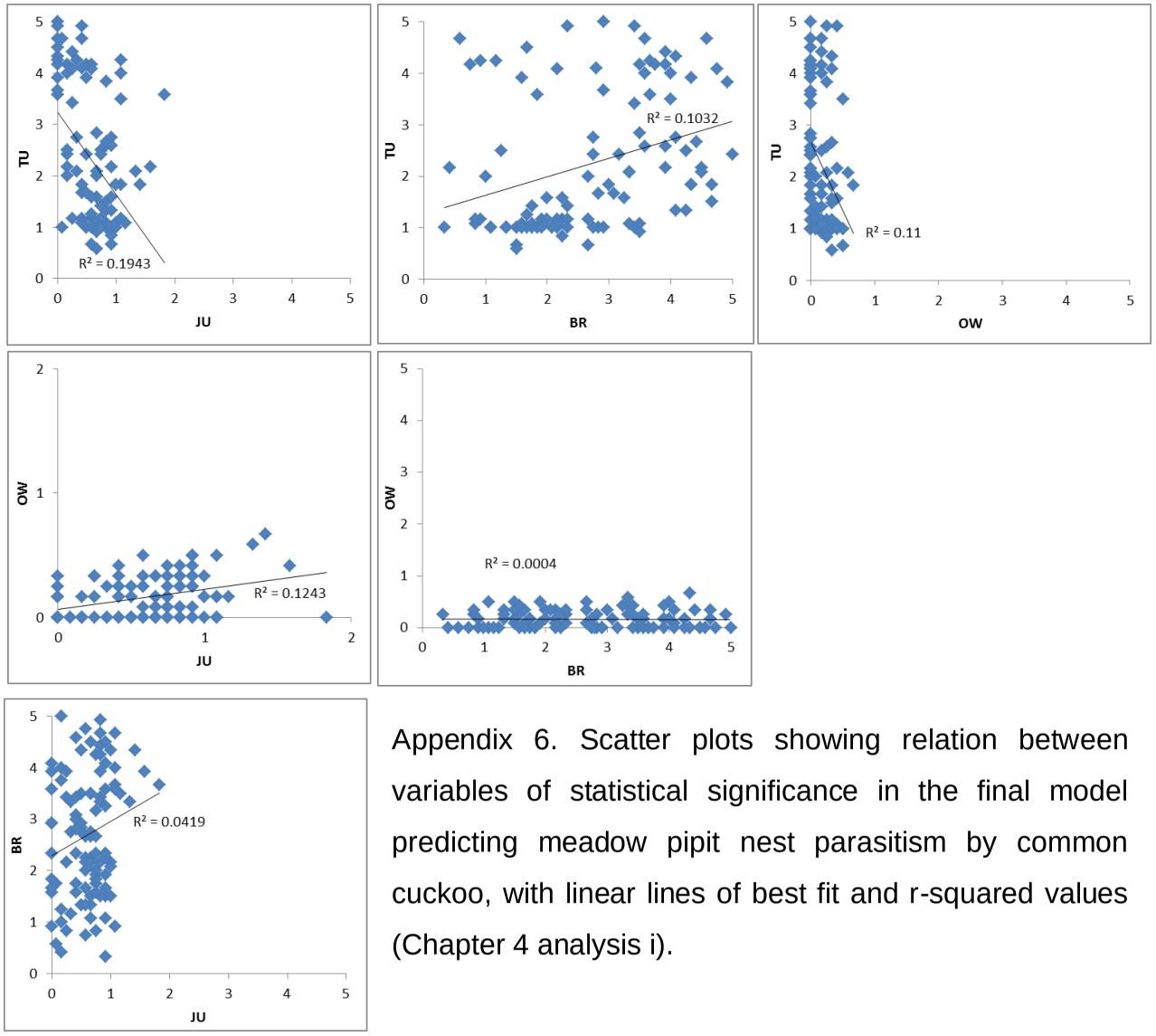
Appendix 3. Plots showing variation in mean prey load size at unparasitised nests per observation session with nestling age, cloud cover, foraging plot (FP) dead or burnt gorse cover (DG) and FP bracken (BR) (Chapter 3 Table 4).



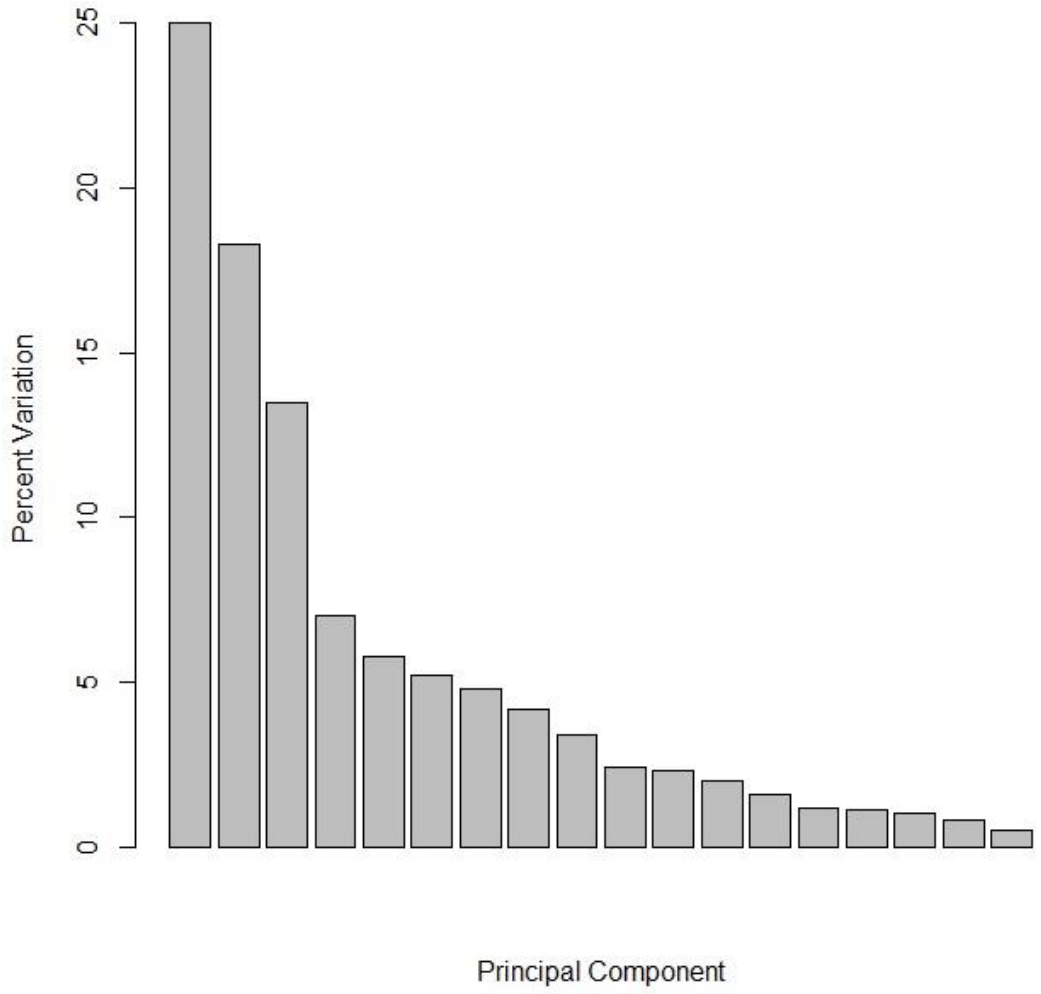
Appendix 4. Plots showing variation in parasitised and unparasitised nests' provisioning visit rate with start hour and duration (Chapter 4 Table 3a).



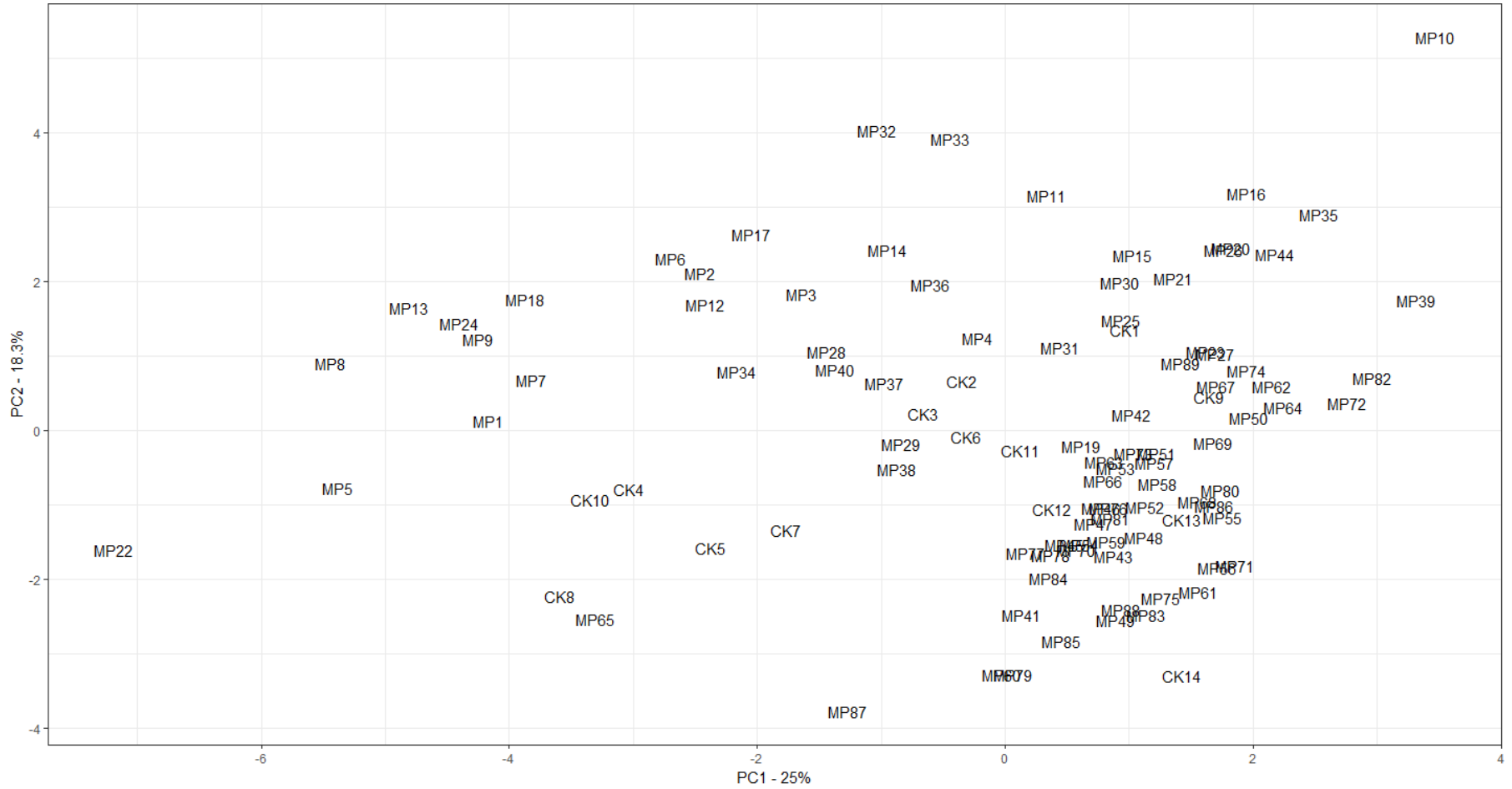
Appendix 5. Plots showing variation in parasitised and unparasitised nests' mean prey load size per observation session with cloud cover, foraging plot (FP) bracken cover (BR) and FP heather cover (HE).



Appendix 6. Scatter plots showing relation between variables of statistical significance in the final model predicting meadow pipit nest parasitism by common cuckoo, with linear lines of best fit and r-squared values (Chapter 4 analysis i).



Appendix 7. Scree plot of principal components of variation in mean habitat cover within 100 m of parasitised and unparasitised nests (n = 103).



Appendix 8. Scatterplot of meadow pipit nests (CK = cuckoo-parasitised, MP = unparasitised meadow pipit nest) for examining clustering of the two types of nest when comparing principal component 1 (PC1) and principal component 2 (PC2) value. Axis title percentages denote % of habitat variation accounted for by each component.

Appendix 9. Months and 10km squares in Devon included for temporal analysis of species occurrence using Rothamsted Insect Survey trapping data 1971-2013.

Species model	Months included	10km British National Grid squares included	Final sample size *n* (no. trapping sessions included)
Garden tiger	Jul-Aug	SS82, SX47, SX69, SX77	3856
Drinker	Jun-Aug	SS82, SX47, SX69, SX77	5620
Magpie	Jul-Sep	SS82, SX47, SX69, SX77	5678
Dotted border	Jan-May	SS82, SX47, SX69, SX77	5751
March moth	Feb-Apr	SS82, SX47, SX69, SX77	3431
Antler moth	Jul-Sep	SS82, SX47, SX57, SX69, SX77	5737
Fox moth	May-Jul	SS82, SX47, SX57, SX77	4447
Winter moth	Jan-Feb and Nov-Dec	SS82, SX47, SX69, SX77	3536
Buff-tip	May-Aug	SS82, SX47, SX69, SX77	7289
Early thorn	Mar-Sep	SS82, SX47, SX69, SX77	11849
White ermine	May-Aug	SS82, SX47, SX57, SX69, SX77	7368
Buff ermine	May-Aug	SS82, SX47, SX69, SX77	7289
Cinnabar	Jun-Jul	SS82, SX47, SX57, SX69, SX77	3706

Appendix 10. Months and subsequent sample size of moth trapping sessions in Devon included for analysis of species occurrence with year and land management type, using Rothamsted Insect Survey trapping data 2003-2016.

Species model	Months included	*n*		
		Sessions	1 x 1 km sites	10 km grid squares
Garden tiger	Jun-Aug	5111	195	34
Drinker	Jun-Aug	5111	195	34
Lackey	Jun-Aug	5111	195	34
Oak eggar	Jul-Aug	3439	164	34
Magpie	Jul-Sep	6489	208	34
Dotted border	Jan-May	4099	115	31
March moth	Feb-Apr	2355	81	29
Antler moth	Jul-Sep	4817	182	34
Fox moth	May-Jul	3122	149	34
Winter moth	Jan-Feb and Nov-Dec	1620	41	21
Buff-tip	May-Aug	6561	208	34
Early thorn	Mar-Sep	9947	231	34
White ermine	May-Aug	6016	194	34
Buff ermine	May-Aug	7939	220	34
Cinnabar	Jun-Jul	4869	185	34

Acknowledgements

Firstly I would like to thank Mark Lawrence, Mark Pennie and Dave Scott, the nest monitoring team at Holne Moor, for contributing a huge amount of time (much of it voluntary) to training me in ‘proper nesting’ and locating most of the hundreds of nests found at that site. Through ringing and crawling around on Dartmoor, respectively, Dave Scott and Stuart Croft contributed hugely in the push to collect adult cuckoo faeces in the field in the final season. Stuart Croft and Malcolm Davies are also owed thanks for collecting a large volume of habitat data and providing excellent company on the moors, during their RSPB research assistant posts. Barry Henwood and Devon Moth Group provided access to decades of moth trapping data, and I extend my thanks to all contributors in the field to that dataset. Additionally Barry Henwood single-handedly and expertly identified all of the cuckoo prey in the photographic analysis. I thank Kevin Rylands at RSPB and the team at DevonBirds for providing the cuckoo data for chapter 6. Sara Zonneveld was responsible for most of the samples and measurements collected from nestlings and provided great practical support. Anke Lange provided an immeasurable amount of practical support, one to one training, time and patience in the laboratory work that provided all the cornerstone diet data for this thesis. Kind access permission was given by landowners Kevin Cox, Patrick Bugg, Heather Parr, and the Meldon Commoners Association. Additionally I owe Andy King, Karen Moore, Hazel Knipe and Paul O’Neill a huge thanks for guidance regarding the DNA sequencing. Harbour provided much needed company on several excursions to the field.

My supervisory team have been second to none and I thank Charles, Jeremy and Stuart for all offering an open door, a great deal of time and patience and a massive amount of one to one training, advice and discussion. I look forward to working with you all on the papers from this study! Thank you to NERC and the RSPB (and its members) who jointly funded this project. Last but not least I owe a great debt of gratitude, time and attention to Emzi Frater for the huge amount of practical advice, support and motivation she has provided from planning to the end of this thesis.